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THYROID AUTOANTIBODIES IN THE FAMILIES OF CRETINS*

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ABSTRACT

A test was made of the hypothesis that thyroid autoantibody in the mother is transmitted through the placenta to combine with fetal thyroid and produce intrauterine fetal hypothyroidism. The sera of 56 members of 20 families containing cretins, and 21 members of 12 control families of comparable age were studied with the use of the tanned red blood cell hemagglutination technique of Boyden. All sera yielding a positive reaction were also tested with complement fixation and precipitin techniques. In 3 out of 20 mothers of cretins the titers were positive on repeated testing with the tanned red blood cell technique. There was no cross reaction with human liver or kidney. No demonstrable titers were found in the sera of cretins or their fathers or siblings. None of the tested mothers of cretins had a history of thyroid disease. In only 1 mother of a control family were serum titers positive. In this case there was a history of thyrotoxicosis treated with radioactive iodine six months previously. The results of this study fail to support the hypothesis that transplacental transfer of thyroid autoantibody from mother to fetus is a cause of congenital hypothyroidism.

THE cause of thyroidal defects resulting in cretinism is unknown. At least some cretins are born without the distal femoral epiphyseal and talus bones which are normally present at birth. This evidence of retarded bone maturation in cretins at birth suggests that the fetus has suffered from

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lack of thyroid hormone in utero. Rose and Witebsky (1) produced morphologic changes similar to those in human Hashimoto's struma, in the thyroid glands of rabbits by active immunization of the animals with rabbit thyroid extracts. Roitt *et al.* (2) demonstrated thyroid autoantibodies in the sera of patients with Hashimoto's disease. Doniach and Roitt (3) found thyroglobulin precipitins in the sera of 6 patients with "spontaneous" nongoitrous myxedema.

In view of these considerations it seemed logical to investigate the possibility that thyroid autoantibody in the mother might be transmitted through the placenta to combine with the developing fetal thyroid and produce intra-uterine hypothyroidism (4). We report here our studies of the sera of 20 families with cretinous children and 12 families with no cretinous children.

METHOD

Sera tested

Sera were tested from 20 mothers of cretins, 10 fathers of cretins, 16 cretins and 8 unaffected siblings. Controls consisted of sera from families without cretinism. The control families, seen in the Pediatrics Clinic because of diabetes, included 9 mothers and 12 children of comparable age and sex distribution. The cretins ranged in age from 1 month to 10 years, and the mothers from 27 to 68 years, at the time the blood specimens were secured. The diagnosis of cretinism was proved in a comprehensive survey (5) utilizing a complete medical history, physical examination, thyroidal 1-, 2-, 5- and 24-hour I^{131} uptakes (6), and serum levels of PBI^{131} (7), cholesterol (8) and organic iodine (9) after omission of thyroid medication for six weeks, and twenty-four hours after a subcutaneous dose of 10 units of thyrotropin (TSH).[1] Charts containing data accumulated in earlier admissions to other hospitals, and baby books were also reviewed. The mother, father and siblings were similarly studied when there was any suggestion of thyroid disease in the parents on the bais of the medical history.

Serology

Blood was centrifuged immediately after clotting and the sera were frozen at $-10°$ C. Thyroid antigen was prepared by the method of Witebsky and Rose (10) from human exophthalmic goiter obtained at the time of surgical thyroidectomy. Witebsky's modification of Boyden's tanned red blood cell hemagglutination technique (10) was used initially to demonstrate the presence or absence of circulating thyroid autoantibodies. All sera yielding positive results were retested. Such sera were also tested against human liver and kidney extract prepared in a fashion identical to that used for preparing thyroid antigen. Titers are expressed as reciprocals of the greatest dilution of serum clearly producing agglutination. Sera yielding positive reactions with the tanned red blood cell hemagglutination technique were also tested using complement fixation (11) and precipitin (12) techniques.

RESULTS

The tanned red blood cell technique revealed the following titers on different days in the sera of 3 mothers of cretins: positive to +81 and +243 in

[1] Armour's Thytropar.

one mother, to +24 and +3 in the second mother, and to +768 and +1536 in the third mother. These sera showed no reaction with human liver or kidney extract, and complement fixation and precipitin tests yielded negative results. These 3 mothers of cretins, aged 40, 40 and 55 years, exhibited no evidence of past or present thyroid disease. In their cretinous children, aged 9, 9 and 9 years respectively, there was no reaction to serologic testing.

By the tanned r.b.c. technique, the serum of 1 mother in the control group also showed positive reactions on different days (+96 and +192). This mother was 51 years of age and had been treated with I^{131} for toxic goiter six months previously; she was euthyroid at the time of this study. Her daughter was 14 years old and her serum gave a negative reaction.

DISCUSSION

There is adequate evidence that maternal antibodies can be transmitted through the placenta to the fetus (13, 14). Brown (13) vaccinated women against poliomyelitis during the seventh and eight months of pregnancy. Serum specimens were obtained from the mother before vaccination, at the time of delivery, from umbilical cord blood, and from the infant at twenty-four hours, one week, one month, two months and three months after birth. Statistical analysis of the serum titers of mothers at the time of delivery, of the umbilical cord blood, and of the infant at twenty-four hours showed that the poliomyelitis antibody content of these 3 specimens was essentially the same. The titers of passively transferred antibodies in the infants declined with age at the same rate, regardless of whether they had been stimulated naturally by infection or artificially by vaccine. Many infants had demonstrable antibodies at the third month of life but the duration of antibody was a direct function of the height of the antibody titer in the mother at the time of delivery.

There is also good evidence that humans can develop circulating thyroid autoantibodies in disease states such as Hashimoto's struma (2, 3). The mechanism here is postulated to be sensitization to stored thyroid hormone (thyroglobulin) that gains entrance to the circulation by trauma to the neck, surgical thyroidectomy, I^{131} thyroidectomy, or some unknown abnormality peculiar to Hashimoto's disease. Martin and Stanbury (15) and Lashmet, Gurney and Beierwaltes (16) observed rare but unusually marked responses to TSH. It is conceivable that ordinary stimuli to TSH production, such as iodine deficiency and ingestion of goitrogens, might cause dumping of stored thyroglobulin into the circulation and resultant development of thyroid autoantibody in the rare person who is unusually sensitive to TSH.

In spite of these theoretical considerations, the present study fails to support the hypothesis that transplacental transfer of thyroid autoantibody from mother to fetus is a cause of congenital hypothyroidism.

Acknowledgments

We are grateful to Misses Kay Tanner and Norma Spafford for their valuable assistance with this study.

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EFFECT OF NORETHANDROLONE ON 17-KETOSTEROID EXCRETION IN PROSTATIC CANCER PATIENTS

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ABSTRACT

Norethandrolone (19-nortestosterone, 17α-ethyl) was administered to 5 men with histologically proven, actively progressing, disseminated prostatic cancer, after individual daily levels of urinary 17-ketosteroid excretion had been established. Each patient received 100 mg. of norethandrolone daily for periods varying from one to three months. The 17-ketosteroid determinations were repeated at intervals of three to four weeks.

Norethandrolone provided palliation in 4 of the 5 patients, consisting chiefly of relief of pain, improved appetite, and significant gain in weight. No objective changes were noted in prostatic size and induration, in the number and size of osseous metastases, or in the levels of serum acid and alkaline phosphatase. As contrasted with the usual effect of testosterone, the administration of norethandrolone was consistently followed by significant reductions in urinary total neutral 17-ketosteroids. This response was observed in the 3 patients who had previously been castrated as well as in the 2 who had not been castrated. The decrease affected all of the less polar 17-ketosteroids uniformly (androsterone, dehydroepiandrosterone and etiocholanolone). No consistent changes were noted in the levels of 11-keto- or 11β-hydroxysteroids. The results of this study tend to confirm earlier observations that the presence of an alkyl function at the 17-position interferes with the formation of 17-ketosteroids, perhaps by preventing the complete conversion of testosterone to 4-androstene,3,17-dione, or by interfering with the production of dehydroepiandrosterone. Supplementary animal experiments have also shown that norethandrolone partially inhibits the full effects of administered testosterone on the prostate of both intact and castrated male rats.

In view of the fact that norethandrolone exerted no apparent effect on the course of the prostatic cancer in the presence of significant reductions in the excretory levels of androgenic 17-ketosteroids, it seems difficult to accept the hypothesis held by a number of investigators that androgenic 17-ketosteroid values constitute an index of prostatic cancer activity.

IT HAS been known for a number of years that some cases of actively-progressing, disseminated prostatic cancer that have relapsed following castration and estrogen therapy may be benefited temporarily by administration of testosterone (1). Improvement is limited to such subjective

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manifestations as pain, appetite, general strength and well-being, and to certain objective but nonspecific criteria, namely, body weight and the hematologic picture. These changes are less dramatic than those which follow castration or estrogen therapy in previously untreated patients, and they are unaccompanied by objective evidence of regression of the primary growth or metastases. Their occurrence in the presence of the rapid and sustained elevation of the serum acid phosphatase level also induced by testosterone, indicate that they are essentially anabolic in nature, rather than the result of a specific anti-tumor property of the steroid. Unfortunately, however, in a certain number of patients with prostatic cancer, testosterone exacerbates the disease to an alarming degree, thereby nullifying its anabolic advantages. Thus its use in cases of relapse must be undertaken with considerable caution.

With the recent availability of steroids possessing more favorable anabolic: androgenic ratios than testosterone itself, it has become possible to extend the investigation of this therapeutic approach. One of the more promising compounds of this type is the testosterone derivative, norethandrolone, or 19-nortestosterone, 17α-ethyl, (Nilevar[1]). Norethandrolone has been extensively studied, and is currently being used in a variety of debilitating diseases (2). It is approximately as anabolic as testosterone, but is much less androgenic, judging from available clinical and experimental evidence (3, 4).

Accordingly, a clinical study was designed to assess the effects of this compound in patients with disseminated prostatic cancer, in whom the disease had relapsed following previous castration and estrogen therapy. Because norethandrolone exhibits a small but definite degree of androgenicity, patients were selected who were regarded as otherwise hopelessly incurable.

A preliminary report on the clinical effects of norethandrolone in the foregoing patients was made in 1957 (5), and these findings will be reviewed briefly in the section on Clinical Observations. The chief purpose of this paper, however, is to describe in some detail the effects of norethandrolone on the urinary excretion of 17-ketosteroids.

METHODS

Five patients with histologically proven, actively-progressing, disseminated prostatic cancer were chosen from the Second (Cornell) and Fourth (New York University) Urological Services of Bellevue Hospital. Three of them had been castrated. Upon admission to the study, all medication was discontinued which might conceivably alter the response to the experimental compound. Urine specimens were collected by catheter for periods ranging from forty-eight to one hundred and twenty hours, in order

[1] The authors wish to thank Drs. Irwin C. Winter and Robert L. Craig of G. D. Searle & Co., Chicago, for providing the Nilevar used in the study.

that a minimum of 30 mg. of 17-ketosteroids per patient would be available for assay.

The pooled specimens from each patient were subjected to preliminary beta-glucuronidase hydrolysis, followed by continuous ether extraction. The remaining material was acidified (pH 0.8) and again extracted with ether. The ketonic fraction of the 17-ketosteroids was further separated into its major components by column chromatography (alumina) using the gradient elution method of Lakshmanan and Lieberman (6). The principal steroids measured were dehydroepiandrosterone, androsterone and etiocholanolone; these appear in the chromatographic patterns as peaks II, III, and IV (Figs. 1–6). The 11-oxygenated steroids are designated simply as peaks V (11-keto) and VI (11-β-hydroxy). No attempt was made to resolve the latter groups further, because of interference with their colorimetric determination by unchanged norethandrolone appearing in the eluates (see section on Steroid Studies).

Pre-treatment 24 hour total and individual 17-ketosteroid levels were established in each patient, following which administration of norethandrolone was begun. All patients received 100 mg. daily, and were maintained on this regimen for periods varying from one to three months. The 17-ketosteroid studies were repeated at intervals of three to four weeks.

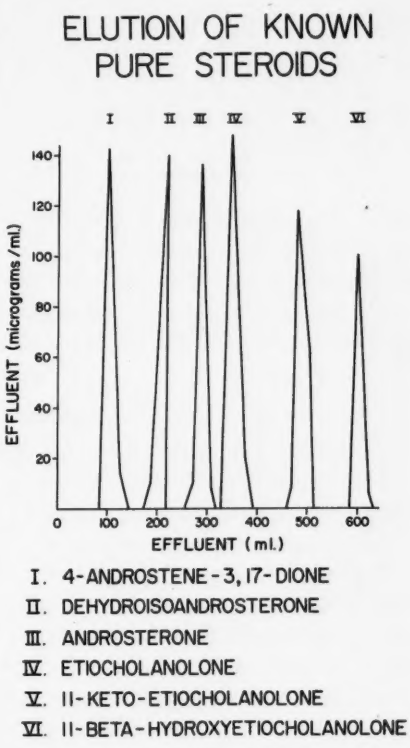


FIG. 1. Chromatographic pattern of urinary steroid metabolites.

CLINICAL OBSERVATIONS

Four of the 5 patients who received norethandrolone were generally improved, in that they experienced definite relief of pain (usually within four days), increased appetite and strength, and a sense of well-being. An appreciable gain in weight was recorded in each instance, the largest being an increase of 46 pounds in a man who had weighed 100 pounds at the time of admission to the study. The quality of the clinical response in these patients was not related to the nature of the response to earlier therapy; nor did the presence or absence of testes seem to be a factor. The only patient who failed to respond (Case 1) had been under treatment for seven years for known, progressive prostatic cancer and was critically ill when norethandrolone was started. His downhill course was unaffected by the drug and he died one month later. No toxic reactions were noted in any of the patients.

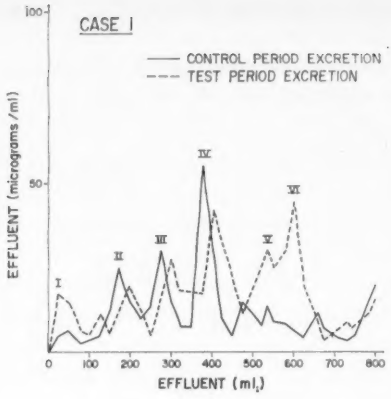


FIG. 2

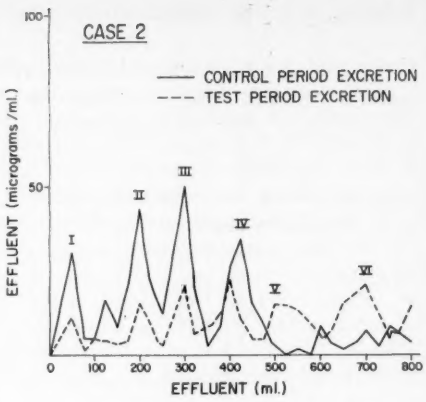
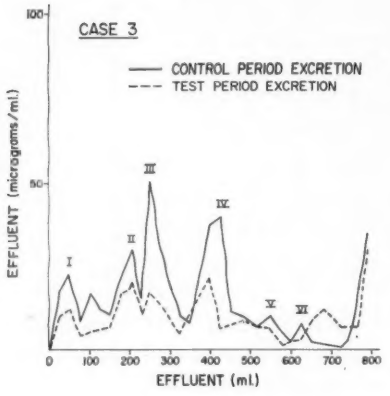


FIG. 3



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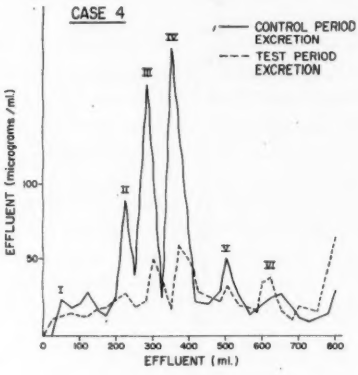

FIG. 5

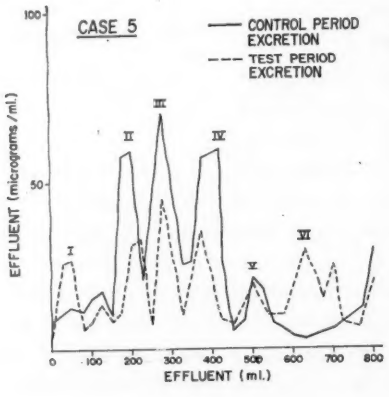

FIG. 6

FIGS. 2–6. Effect of norethandrolone on the chromatographic pattern of urinary steroid metabolites in Cases 1–5.

TABLE 1. EFFECT OF NORETHANDROLONE ON URINARY EXCRETION OF TOTAL NEUTRAL 17-KETOSTEROIDS

Patient	Pre-treatment 17-KS (mg./24 hrs.)	Post-treatment 17-KS (mg./24 hrs.)	Reduction (%)
A. (Case 1)	5.02	2.85	43.2
G. (Case 2)	4.51	2.07	54.2
H. (Case 3)	5.66	3.70	34.6
D. (Case 4)	7.67	2.34	69.5
T. (Case 5)	6.08	3.94	34.9

There were no detectable changes in prostatic size or consistency. Osseous metastases remained similarly unaltered. Serum acid phosphatase levels were unaffected, whether initially elevated or not; this is in direct contrast to the usual response to testosterone. The serum alkaline phosphatase level, which was initially increased in all patients, changed little, if at all.

STEROID STUDIES

In contrast to testosterone, the administration of norethandrolone was consistently followed by significant reductions in the 24-hour urinary excretion of total neutral 17-ketosteroids (Table 1). This response was the same in the castrated (Cases 1, 2 and 3; Figs. 2, 3 and 4) and in the noncastrated men (Cases 4 and 5; Figs. 5 and 6). Furthermore, the decrease affected all of the principal (less polar) 17-ketosteroids uniformly (Table 2 and Figs. 2–6). Changes in the 11-keto- and 11,β-hydroxysteroids were not consistent. Little significance, however, can be attached to alterations in the excretory levels of these two groups of steroids following administration of norethandrolone, because early in the study it was found that unchanged norethandrolone, appearing in the urine, was eluted with the more polar

TABLE 2. EFFECT OF NORETHANDROLONE ON STEROID COMPONENTS OF URINE
(mg./24 hrs.)

	Fraction	Patient A.		Patient G.		Patient H.		Patient D.		Patient T.	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Less polar	DHA	0.60	0.28	0.98	0.23	0.80	0.57	0.83	0.22	1.20	0.37
	Andro.	0.95	0.33	1.11	0.24	1.63	0.63	1.74	0.41	1.84	0.70
	Etio.	1.44	0.76	1.23	0.37	1.35	0.57	2.00	0.44	1.24	0.54
More polar	11-Keto	0.46	0.43	0.22	0.13	0.30	0.24	0.86	0.29	0.48	0.53
	11-OH	0.45	0.57	0.19	0.19	0.26	0.16	0.68	0.34	0.25	0.60
	Unidentified	1.12	0.48	0.78	0.91	1.32	1.53	1.56	0.64	1.07	1.22

steroids and interfered with the Zimmermann reaction. For this reason, also, no attempts were made to separate these groups of steroids into their respective epimers.

DISCUSSION

In the 5 cases studied, it was found that the daily excretion of androsterone and etiocholanolone decreased during the period of treatment. Norethandrolone itself would not be expected to contribute to the 17-ketosteroid pool, inasmuch as there exists fairly good evidence that oxidative

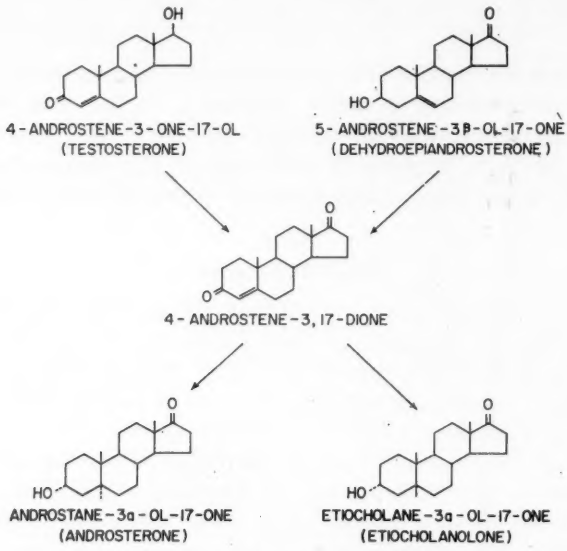

FIGURE 7

cleavage does not occur at the 17-alkyl-17-hydroxy position (7–11). The 17-alkyl group in norethandrolone may similarly act to prevent the complete conversion of testosterone to 4-androstene-3,17-dione, from which androsterone and etiocholanolone arise (12–15). This may account, in part, for the observed reduction in these two steroids in the noncastrated patients.

There is another possible explanation, however, for the decreased levels of androsterone and etiocholanolone, which applies to the castrated as well as to the noncastrated patients. Both of these steroids have been shown to arise from 4-androstene-3,17-dione, which, in turn, originates not only from testosterone but also from dehydroepiandrosterone (16–18) (Fig. 7). Brooks and Prunty have shown that norethandrolone does not interfere

with the conversion of the 4-androstene compound to androsterone and etiocholanolone (19). This, taken together with the reduction in dehydroepiandrosterone observed in our study and in that of Brooks and Prunty, may signify that the reduced production of dehydroepiandrosterone also contributes to the drop in androsterone and etiocholanolone.

The reduction in dehydroepiandrosterone excretion, in turn, may be 1) direct, due to inhibition of adrenocortical function by norethandrolone (19), or 2) indirect, due to suppression of pituitary tropic hormones, *i.e.*, LH (7)

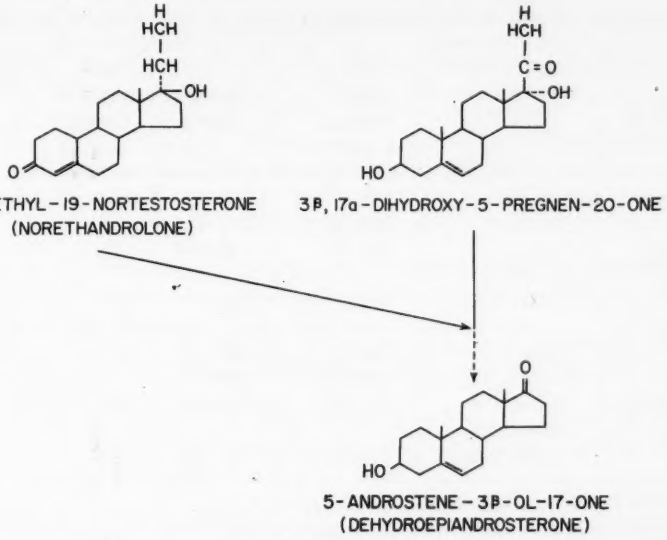

FIGURE 8

or ACTH (8), by this steroid. With respect to the first hypothesis, it is possible that norethandrolone competes in the adrenal for enzyme systems normally active in the conversion of natural precursors to dehydroepiandrosterone. Although the mechanism and intermediates in the biosynthesis of dehydroandrosterone have yet to be elucidated, *in vivo* studies in man (20) suggest that it may arise from 3β,17α-dihydroxy-5-pregnen-20-one, a compound which has been isolated from the urine of patients with adrenocortical carcinoma by Hirschmann and Hirschmann (21). The transformation of the 5-pregnene compound to dehydroepiandrosterone involves rupture of the C17-C20 linkage. Competition by norethandrolone (which is structurally similar to 3β,17α-dihydroxy-5-pregnen-20-one) for an enzyme system involved in conversion of the latter compound, may explain the observed decrease in dehydroepiandrosterone (Fig. 8).

The fact that norethandrolone reduces 17-ketosteroid excretion has led us to wonder whether it may also interfere with the biologic action of endogenous androgens. Preliminary experiments in our laboratory (22) have shown that it seems to inhibit, at least partially, the full effect of administered testosterone in intact and castrated male albino rats (Table 3). This is of interest in the light of recent demonstrations of the key role played by the 17,β-hydroxyl group in the growth-promoting activity of a number of steroids of the androstane series (23–25), for it may mean that

TABLE 3. EFFECT OF NORETHANDROLONE AND TESTOSTERONE ON RAT PROSTATE

Group	Treatment	Mean Body Weight (Gm.)	Range (Gm.)	Mean Prostate Weight (mg.)	Range (mg.)
Intact	Sesame	366 (5)‡	351–398	487	370–916
	Noreth.*	376 (8)	342–401	525	376–682
	Testo.†	369 (8)	345–392	624	474–816
	Noreth.* plus Testo.†	374 (8)	354–405	484	288–650
Castrated	Sesame	352 (8)	320–404	34.7	17.4–45.6
	Noreth.*	339 (9)	317–361	51.4	45.0–60.6
	Testo.†	344 (8)	317–366	170	136–244
	Noreth.* plus Testo.†	360 (9)	346–403	114	92–138

* Total dose over 8-day period, 6.0 mg.

† Total dose over 8-day period, 1.0 mg.

‡ Numbers in parentheses indicate the number of animals in each treatment group.

the full androgenic effect of testosterone depends on whether its 17,β-hydroxyl group can be oxidized to the keto form—an oxidation which norethandrolone appears to depress to some degree.

Norethandrolone exerted no apparent effect on the primary prostatic neoplasm, on the bone metastases or on the levels of serum acid and alkaline phosphatase in any of the patients studied. It is difficult to reconcile the observed reductions in urinary 17-ketosteroids with the view held by some investigators that excretory levels of the androgenic 17-ketosteroids constitute an index of prostatic cancer activity (26, 27). Perhaps long-range clinical studies with norethandrolone are necessary in order to clarify this point. However, should norethandrolone ultimately prove ineffective in arresting the progress of the disease in the presence of sustained reductions in 17-ketosteroid excretion, it may mean that reactivation of prostatic

cancer is due to factors other than stimulation by androgens of nonticular origin.

Acknowledgments

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THE ROLE OF HORMONES IN HUMAN BEHAVIOR. I. CHANGES IN FEMALE SEXUALITY AFTER ADRENALECTOMY

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ABSTRACT

Twenty-nine women, mean age 51 years, who had undergone total bilateral oophorectomy and adrenalectomy for metastatic breast cancer were interviewed about changes in their sexual desire, activity and responsiveness at an average period of twelve months after adrenal ablation. Of the 17 patients reporting some sexual desire preoperatively, 14 experienced a decrease after surgery, the majority losing all desire. Of the 17 patients sexually active preoperatively, all reduced their frequency of intercourse postoperatively, almost half stopping entirely. Of the 12 patients responsive in intercourse preoperatively, 11 experienced a decrease after surgery, almost all becoming completely unresponsive. In each of these categories, the number of patients who lost sexual function entirely was statistically significant. Both sexual feelings and activities declined in all but 2 of the patients not already at zero levels among the 20 who obtained remission of metastatic disease after adrenalectomy, as well as in all but 1 of the 9 patients who did not present objective evidence of benefit from the operation.

Neither breast cancer itself nor any previous surgery had as profound an impact on sexual behavior as did adrenalectomy. In agreement with the preponderance of evidence in the literature, changes in a subgroup of 7 patients who had undergone oophorectomy one to five years before their adrenals were removed indicated that ovarian ablation by itself usually does not radically alter sexual behavior. It was concluded that the adrenal glands and, more specifically, the androgens of adrenal origin, play a critical part in maintaining the patterns of sexual behavior in the human female.

INTRODUCTION

THIS investigation explores the hypothesis that sex hormones of adrenal origin have an important influence on the sexual behavior of women. The adrenal glands are a source of both androgens and estrogens and are probably the exclusive source of androgens in women. Five steroids which possess androgenic activity have been isolated by direct chemical extraction from adrenocortical tissue, but none biologically as active as testosterone, the androgen produced by the male testes (1). Although the adrenal androgens are relatively weak substances, they are produced in greater amounts than the adrenal estrogens.

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In contrast to previous studies of effects of androgens in women, which have been concerned with exogenous hormones, this study examines the results of deprivation of endogenous adrenal hormones. Adrenal ablation, or combined oophorectomy-adrenalectomy, is currently resorted to in an effort to remove sources of estrogenic hormones which are believed to promote or sustain the growth of some types of breast cancer. Recent reviews of the results of adrenalectomy in breast cancer patients omit any mention of effects on sexual behavior (2–4).

MATERIAL AND METHODS

Twenty-nine women who had undergone total bilateral oophorectomy and adrenalectomy for metastatic breast cancer were available for study over the course of a year in the Breast Service Clinic of Memorial Center. The patients varied in age from 31 to 72 years. Only 2 were more than 59 years old, and the mean age for the group was 51 years. Sixteen patients were married, 4 were widowed, 6 were divorced or separated, and 3 were single. Breast cancer had been present from two to fifteen years; the median duration was less than five years. Twenty-two patients had undergone simple or radical mastectomy, and 7 had inoperable breast disease. Seven of the patients had undergone bilateral oophorectomy one to five years before adrenalectomy. All the others were oophorectomized at the time of adrenalectomy.

Intensive interviews at two to thirty-nine months after adrenalectomy (mean interval twelve months, median nine months) probed the pre- and postoperative sexual behavior of these women. Since sexual functioning in the human subject is a complex phenomenon, an attempt was made to differentiate 3 important aspects of such behavior: (a) sexual desire, an appetitive state compounded of innumerable somatic and psychologic elements, (b) heterosexual activity, which can be measured objectively in terms of frequency of intercourse, and (c) sexual responsiveness, which entails a subjective evaluation of the degree of participation in and the gratification obtained from sexual activity.

OBSERVATIONS

The changes in sexual functioning which took place between the time of adrenalectomy and the time of the retrospective interviews are summarized in Table 1. Before adrenalectomy, 8 of the patients had no desire, no sexual activity and no responsiveness, and they experienced no changes after the operation. Four women were sexually inactive before adrenalectomy although they felt sexual desire, and 4 others engaged in sexual relations without sexual desire.

Changes in sexual desire

Seventeen of the women reported some level of conscious sexual desire prior to adrenalectomy, whereas 12 were aware of no such feelings. Of the 17 with sexual desire, 3 patients reported no change after the operation, but 14 experienced a noticeable decrease in desire. Of the 14 who reported a decrease, 10 experienced a total loss of desire, and in the remaining 4 it was

TABLE 1. SUMMARY OF CHANGES IN SEXUAL BEHAVIOR AFTER OOPHORECTOMY AND ADRENALECTOMY

Type of change	Number of patients experiencing change		
	In sexual desire	In sexual activity	In sexual responsiveness
Maintained preoperative level	3	0	1
Decreased from preoperative level	14	17	11
Some decrease	4	10	2
Decrease to zero	10*	7†	9*
Unchanged, zero level	12	12	17
Totals	29	29	29
Percentage of those not at zero level preoperatively who experienced decrease	82%	100%	92%

* Number decreasing to zero significant at .01 level.
† Number decreasing to zero significant at .05 level.

considerably reduced but not absent. The number changing from a state of some desire to no desire is significant at the .01 level.

Changes in sexual activity

Seventeen women were sexually active prior to adrenal surgery. All decreased their sexual activity afterward. Seven discontinued sexual activities altogether, and the other 10 reduced the frequency of intercourse. The number changing from some activity to no activity is significant between the .02 and .05 levels.

Three patients received testosterone after adrenalectomy. Two were in a terminal condition; the third reported some heightening of desire and increase in frequency of intercourse during testosterone therapy.

Changes in sexual responsiveness

Twelve patients reported some pleasurable response in their sexual relations before adrenalectomy. After adrenalectomy, 9 of the 12 reported total loss of responsiveness, 2 experienced a decrease, and in 1 there was no change. The number changing from some responsiveness to none is significant at the .01 level.

A 54-year-old patient, interviewed almost two years after oophorectomy-adrenalectomy which was followed by remission of metastatic breast cancer, illustrates the dramatic changes which can occur:

"My husband is very patient and understanding . . . There was no change in my sex life after the first operation (mastectomy). My feelings were just what they had been before. Immediately after the second operation (combined oophorectomy-adrenalectomy), I noticed a change. There was a loss of interest and desire for sex. There was no pleasure in it whatsoever. Maybe I didn't try, but I just didn't feel any desire for it. Before the operation (combined procedure) I never had any trouble reaching an orgasm. I had one almost every time. There was no change after the breast operation; I would even get desire from reading a book or something like that. After the breast operation, there was no loss of interest on my husband's part either, and there was no special cautiousness on either of our parts. Now my husband is still very considerate, but I have no desire or pleasure in it at all, none at all."

Three patients experienced a subtle shift in emphasis from genital gratifications to the affectionate and tender satisfactions of sexual relationships and to the reassurance to be gained from close body contact.

Relationship between physical condition and sexual changes

The fact that all the patients included in this study had a fatal disease requires that the sexual behavior of those who benefited from adrenalectomy be compared with the behavior of those who did not obtain relief of symptoms or remission of disease. The 4 patients for whom adrenalectomy was without benefit experienced decreases in all sexual manifestations not already at a zero level. The 5 patients who obtained some palliation of symptoms without objective evidence of improvement experienced decreases in all sexual measures which were not already zero, with the exception of 1 woman who maintained her level of desire but became sexually inactive.

There were 20 patients who presented objective evidence of improvement. This proportion of patients benefited by adrenalectomy is greater than usually reported, since this research unavoidably excluded some patients who obtained little or no benefit. In 6 of the 20 improved patients, all sexual variables were at zero levels prior to adrenal ablation. In 12 of the remaining 14 improved patients, there was a postoperative decrease in all variables not already at zero levels; in only 2 of the 14 was there maintenance of some element of the sexual functioning at the preoperative level. Of these 2 women, one maintained sexual desire but had already become sexually inactive, and the other maintained desire and responsiveness but decreased sexual activity after surgery.

Every one of these 20 patients who was sexually active before adrenalectomy reported a decrease in sexual activity notwithstanding observed improvement in her general physical condition. These decreases in sexual functioning reported by this majority of the subjects in the study cannot be attributed to progression of the mammary cancer.[1]

[1] Many factors besides general state of health may influence sexual behavior, such as individual sex anatomy, sexual technique, sexual inhibitions, the interpersonal climate

Effects of oophorectomy

In 7 of the 29 cases, the ovaries had been removed sixteen to sixty months before adrenalectomy. Table 2 presents a comparison of the effects of oophorectomy with the effects of subsequent adrenalectomy in this subgroup. All 7 of these patients reported having some sexual desire before oophorectomy. Five were sexually active, and 2 were sexually inactive

TABLE 2. COMPARISON OF EFFECTS ON SEXUAL BEHAVIOR OF OOPHORECTOMY AND ADRENALECTOMY (INTERVAL BETWEEN OPERATIONS RANGED FROM 1 TO 5 YEARS)

Effect	Number of patients experiencing change					
	In sexual desire		In sexual activity		In sexual responsiveness	
	After ovarian ablation	After adrenal ablation	After ovarian ablation	After adrenal ablation	After ovarian ablation	After adrenal ablation
No decrease	2	0	2	0	2	0
Some decrease	4	2	3	2	3	1
Decrease to zero	1	4	0	3	0	4
Unchanged, zero level	0	1	2	2	2	2
Totals	7	7	7	7	7	7

widows. After ovarian surgery, 1 of these patients lost all her sexual desire, 4 experienced a decrease in desire, and 2 reported no change. Two of the women who experienced a decline in desire had undergone oophorectomy

between partners, and the desire for or fear of pregnancy. Most such variables are not likely to have changed fundamentally during the periods in the lives of these women from which comparisons were drawn for this report, namely, the immediate pre-adrenalectomy period versus the post-adrenalectomy period. The patterns of sexual behavior which might have been affected by these variables were largely set down many years previously. Although the impossibility of pregnancy after combined ovarian and adrenal ablative surgery might be expected to increase sexual activity in certain patients; this result was not observed. Nor did any patient indicate that inability to conceive had a directly inhibitory effect.

The patients were suffering from widespread metastatic disease at the time the decision was made to resort to adrenalectomy, and most of them felt better after this operation than they had felt just before the operation. Remissions eventually come to an end and the disease again runs a downhill course, but terminal stage conditions were not used in this comparative study. Trends in sexual functioning throughout the lives of the individual patients were reviewed. The data cannot be presented here in detail, but, briefly, they indicated that neither the discovery of breast malignancy nor any previous surgery had the negative impact on sexual behavior that adrenal ablation had.

combined with hysterectomy. Recent reports on this combined operation indicate that subsequent disturbances in sexuality may be related to irrational fears and distorted beliefs concerning the loss of the uterus and the effects of surgery in the genital area (5, 6). After adrenal ablation, 4 of the patients who had retained some desire after oophorectomy lost all desire and the other 2 patients experienced decreases in desire.

Of the 5 patients who were sexually active at the time of oophorectomy in this subgroup of 7, 2 reported no changes in frequency of intercourse; the other 3 reported decreases, but not discontinuance of intercourse after their ovaries were removed. After adrenalectomy, of the 2 patients unaffected by oophorectomy, one experienced a decrease in frequency of intercourse and the other ceased having sexual relations. The 3 patients whose sexual activity had declined after oophorectomy all experienced further declines, 2 of them discontinuing sexual relations. Changes in sexual responsiveness in this subgroup closely paralleled changes in sexual activity.

There was a decrease to zero of at least one sexual variable in 1 out of 7 patients after ovarian surgery and in 5 out of 6 patients after subsequent adrenal surgery. In none of 5 sexually active patients did all three sexual variables decrease to zero after ovarian ablation; however, this did occur in 2 of the 5 patients after adrenal ablation.

DISCUSSION AND CONCLUSIONS

From the findings of this study, it is apparent that any discussion of the hormonal bases of human female sexuality must concern itself as much with the adrenal glands as with the ovaries. Since the ovaries secrete by far the greater proportion of the endogenous estrogens in the female circulation, their removal or the reduction of their function (as in the menopause) deprives a woman of the major source of estrogens. A survey of recent studies (7) indicates that radical changes in the sexual behavior of women do not follow either surgical castration or the menopause. For example, Filler and Drezner (8) found that among 41 women under the age of 40 whose ovaries were removed, 36 (88 per cent) experienced no change in sexual libido. Kinsey *et al.* (7), reporting on 123 women who had their ovaries removed, confirmed the general opinion that there is no modification of sexual responsiveness or capacity for orgasm which can be clearly identified as due to ablation of the ovaries. Although deprivation of ovarian hormones has little effect on sexual behavior, evidence has been presented in this paper for a close relationship between loss of adrenal hormones and disruption of sexual behavior in women.

The gonads and the adrenals together, activated by the pituitary, may be considered to provide the hormonal basis for human female sexuality; but the major nonreproductive component, the erotic component, is here

attributed to the adrenal hormones. The evidence points, furthermore, to the adrenal *androgens* as important hormonal contributors to sexuality in the human female. The adrenal glands are probably the exclusive source of androgens in the intact female. After adrenalectomy, urinary androgen excretion falls to very low levels, whereas after castration or the menopause it is not reduced (1). Dorfman and Shipley state that no vital function has been definitely established for adrenal androgens in the female other than promotion of the growth of pubic and axillary hair (1); nevertheless, the present investigation indicates another important function, namely, that of promoting and sustaining certain aspects of female sexual functioning.

On the behavioral side, the evidence reviewed here indicates that loss of the major source of estrogens does not by itself critically alter the sexual expression of women who have previously achieved good sexual adjustment. The patients in this study, all of whom had undergone both oophorectomy and adrenalectomy, may be considered to have been deprived of their endogenous androgens. It has been shown that their sexual functioning underwent significant alteration in the direction of decrease or total loss of sexual desire and responsiveness, and decrease or cessation of sexual activity. Thus the adrenal glands rather than the ovaries, and the *androgens* supplied by the female adrenals, rather than the estrogens, are implicated in this remarkable decrement in manifest sexuality.

There are certain objections which may be raised to this conclusion. After oophorectomy, estrogens may still be present (9, 10). Since there are apparently enough left to affect the growth of estrogen-dependent breast cancer (though the dependence of cancer growth on these hormones is only presumptive), the influence of estrogens on sexuality cannot be ruled out entirely. Metabolic transformation of massive doses of testosterone propionate to small amounts of estrogen has been shown to occur in women whose gonads and adrenals have been removed (11). Estrogens produced by the adrenal glands or derived metabolically from adrenal androgens may sustain sexual functioning after oophorectomy. Small quantities of estrogens may be sufficient to maintain habitual levels of sexual behavior. If this be true, adrenalectomy may reduce sexual desire by removing the secondary source of estrogens. If not, it must be concluded that loss of adrenal *androgens* accounts for the sexual changes after adrenalectomy.

Evidence from studies on the clinical administration of sex hormones bears most tellingly in favor of the conclusion that it is the androgens which are primarily involved. Estrogens administered to women with gynecologic disorders or cancer do not usually have an effect on sexual desire (12, 13), nor have estrogens proved effective in the treatment of frigidity (1,

14). However, numerous reports dealing with the therapy of gynecologic disorders and cancer have established that exogenous *androgens*, chiefly testosterone propionate, do have a heightening effect on libido in many women, including some who lacked or had only minimal desire before (12, 15–18). One patient in this study who was subsequently given a course of testosterone therapy experienced a noticeable increase in sexual desire and activity.

Various explanations have been advanced regarding the mechanism by which androgenic hormones might influence female sexuality. One attributes the effects to increased vascularization of the vulval region, with sensitization of the clitoris (12, 14). Another regards the metabolic stimulation attendant upon androgen intake to be the essential physiologic change, the increased libido being secondary to the generally increased well-being (7). Still another point of view assigns importance to increased psychic sensitization and susceptibility to psychosexual symbolism under the control of neurohumoral mechanisms in the central nervous system (12, 19). These hypotheses are by no means mutually exclusive.

What of the few exceptional patients in whom ovarian and adrenal ablation did not cause a decrease in sexual desire, and what of those who continued to experience some reduced measure of desire? Defensiveness about the waning of highly prized feminine attributes or the failure of sexual powers may cause some women to report retention of desire when they actually do not have it. Accessory adrenal tissue (20) or possibly food ingested by the patients may supply sex hormones after the combined ovarian-adrenal ablative surgery. Also, after adrenalectomy each patient must have a daily dose of 37.5 to 75.0 mg. of cortisone for preservation of life. The question of whether this cortisone is metabolized to androgenically active substances in significant amounts is controversial on both theoretical and empirical grounds (1, 21–23).

The patients who retained some interest in sexual intimacy with their husbands after adrenal ablation indicated a shift of that interest from the genitally erotic to the more diffusely affectionate gratifications which they derived from the tenderness and reassuring physical closeness in sexual relations. This affectionate component of sexual activity may be sustained not by hormonal but rather by psychologic factors. One patient who before adrenalectomy had dreams of intercourse with accompanying orgasm later had dreams involving physical warmth and closeness to another person without genital excitation.

Patterns of sexual expression are not determined exclusively by hormones, nor is sexual behavior entirely determined by learning and training (7, 24). Sex hormones are important in the proper maturation and maintenance of sexual and reproductive mechanisms in the adult human being,

but psychologic factors play a part in setting the levels of intensity and of awareness of the sexual urges and in determining the direction and quality of expression of these urges. The psychologic factors are very intricate. They are developed and modified by the total experience of a person, by relationships with parents and by the social and moral pressures which come to bear on the personality. Disturbances in sexual functioning have been shown in the majority of cases to arise from psychologic causes (14, 25), and they have been favorably influenced by psychotherapeutic intervention. Even when sex hormones are present in intact men and women, there may be no sexual expression because hormonal influences cannot always break through strong psychosocial barriers. On the other hand, even in the state of deprivation resulting from combined oophorectomy-adrenalectomy, sexual feelings and activities may occasionally be sustained by social pressures and by interpersonal needs which are not specifically erotic. Nevertheless, in the majority of cases, deprivation of sex hormones results in loss of interest in the specifically erotic aspects of sexual relationships. The hormones are an essential component among the complex determinants of sexuality.

Under the condition of deprivation of endogenous sex hormones, the adrenal glands have been shown to enter into the determination of human female sexual functioning in a critical manner. There is evidence that adrenal androgens, rather than the estrogens, are the major source or vehicle of this adrenal influence on the sexual behavior of women.

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CONGENITAL VIRILIZING ADRENAL HYPERPLASIA IN IDENTICAL TWINS

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ABSTRACT

Congenital virilizing adrenal hyperplasia in identical female twins 7½ years of age is reported. The diagnosis was based on hypertrophy of the clitoris at birth, progressive virilization and accelerated growth, elevated urinary 17-ketosteroids and pregnanetriol, female sex chromatin pattern and amelioration with corticosteroid therapy. There is only 1 other authenticated example in the medical literature. This is not unexpected, since the anticipated incidence is probably less than 1 per 23 million births. Therapeutic trials with intramuscular injections of a long-acting hydrocortisone preparation, oral prednisone, fluorohydrocortisone and methylprednisolone did not consistently suppress adrenal androgen production but (except for methylprednisolone) did induce Cushingoid phenomena. Cortisone (25 mg.) or prednisone (8 mg.) given intramuscularly every other day successfully suppressed adrenal hyperfunction without causing untoward side-effects.

THE occurrence of congenital virilizing adrenal hyperplasia with pseudohermaphrodism in more than one member of a family is not unusual (1) and suggests a genetic cause for this aberration. Its existence in identical twins, however, is extremely rare and has not been observed by several authorities[1] with wide experience in problems of pseudohermaphrodism.

Rhodes (2) described 1 set of twins and reviewed 8 cases previously reported from 1557 to 1921. Two subsequent examples were reported in 1948 (3) and in 1954 (4); in only the latter case was the diagnosis authenticated.

The present cases are reported both because of their extreme rarity and because they afforded an excellent opportunity to compare the efficacy of several methods of therapy.

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[1] L. Wilkins: Personal communication, Sept. 26, 1955. A. Segaloff: Personal communication, Aug. 16, 1955. L. R. Broster: Personal communication, June 23, 1955. A. Bongiovanni: Personal communication.

CASE REPORT

Eileen and Maureen McG. were born on January 14, 1948, at the Southern Division of the Albert Einstein Medical Center following an uncomplicated full-term pregnancy. The family physician noted enlargement of the clitoris in both babies but, despite his suggestions, further studies were not pursued. At the age of 3 years the clitoral enlargement had increased and the voice had assumed a masculine quality. A few pubic hairs appeared at age 4. Marked acceleration of growth occurred between the fifth and sixth years and permanent teeth began to replace decidual teeth rapidly. Axillary and pubic hair and hirsutism of the arms, legs, upper lip and chin became well marked by the end of the fifth year. There was a progressive increase in virilization and the appearance of facial acne during the sixth and seventh years.

Infantile growth and development had been normal with regard to sitting, standing, walking and talking in comparison with the other siblings. There was no history of birth injury, coma, convulsions, hypoglycemic episodes or shock. The twins had maintained excellent physical health and had chicken pox and measles during their sixth year. Intelligence and school progress were considered to be normal by the parents.

There were no known familial diseases but such traits as hirsutism, deep voices and tallness were common in both the maternal and paternal lines. There was no familial history of congenital deformities or any known endocrinopathies. The father and mother were both 35 years of age at the time of the birth of the twins and were in good physical and mental health. There were 2 siblings, a sister of 13 and a brother of 14 years. Both were entirely normal in growth and development and exhibited no stigmata of endocrine disease. There was no consanguinity. The father was born in Ireland and the mother in the United States.

Physical examination: The twins were admitted to the Southern Division of the Albert Einstein Medical Center on June 6, 1955, when they were 7½ years of age. They were tall, alert, intelligent children whose appearance resembled that of somewhat muscular 12-year-old girls (see Fig. 1, a photograph taken after two months of treatment). Their voices were low-pitched, there were multiple small acneform lesions of the forehead, comedones about the nasal alae, and moderate oiliness of the skin of the face. There was a moderate growth of soft downy hair on the upper lips and prominent sideburns. Axillary and pubic hair was of the adult female type and the axillae contained an adult quantity of sebaceous secretion. There were a few fine colorless striae over the upper anterior thighs. Weight and body measurements are outlined in Table 1.

All permanent teeth except the third molars were fully erupted. There was no palpable breast tissue and the areolae and nipples were prepubertal. The thyroid was normal to palpation and the heart, lungs and abdomen were normal.

In each child the clitoris was markedly enlarged, measuring approximately 5 cm. in length and 2½–3 cm. in diameter (Fig. 2). There was no opening on the dorsal or ventral surface. There was a glans-like cuff at the distal portion of the clitoris from which the thin, somewhat corrugated labia minora took origin. The vaginal introitus and urethra were separate and in normal relationships. The labia majora were normal. The vagina admitted insertion of 1 finger to a depth of ½ inch (Maureen) and 1½ inches (Eileen). Vaginal dilation with a nasal speculum revealed a tiny cervix in each twin.

Maureen's I.Q.[2] was 117 (equivalent to a mental age of 8 8/12 years) and Eileen's I.Q.[2] was 119 (equivalent to a mental age of 8 10/12 years).

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[2] Stanford-Binet, Form L; Wechsler Bellevue Intelligence Scale for Children.

FIG. 1. Identical twins with congenital adrenal hyperplasia after two months of corticosteroid therapy. (Early breast budding and striae on the thighs are results of treatment; pretreatment photographs were technically unsuitable for reproduction.)

FIG. 2. Appearance of the external genitalia in one twin.

TABLE 1. INITIAL BODY MEASUREMENTS AND LABORATORY FINDINGS

	Eileen	Maureen	Norms for 7½ years of age
Height (ins.)	58	58	48.1 ± 2.24
Span (ins.)	53½	56	
Upper measurement, head-pubis (ins.)	29½	30½	
Lower measurement, pubic-floor (ins.)	28½	27½	
Weight (lbs.)	83	80	51.5 ± 8
Eosinophils/cu.mm. (fasting)	531	437	
Blood sugar, fasting (mg./100 cc.)	91	90	
Serum protein-bound iodine (μg./100 cc.)	5.5	5.0	
Urinary tot. neut. 17-ketosteroids, (mg./24 hrs.)*	10.5–20.8	10.6–48.6	Less than 2 mg.
Urinary 17-hydroxycorticoids (mg./24 hrs.)†	0.34	0.16	
Urinary pregnanetriol (mg./24 hrs.)‡	70	64	Less than 1 mg.

* Method of Drekter *et al.* (5).
† Method of Reddy *et al.* (6).
‡ Method of Bongiovanni (7).

STUDIES

In Table 1 initial laboratory studies are charted. The hemogram, urinalysis and blood sugar findings were within normal limits. The urinary 17-ketosteroids were elevated and the corticoids were low. Pregnanetriol was present in large amounts. Urinary gonadotropin was moderately elevated in a single determination.

An x-ray survey disclosed markedly advanced skeletal maturation. The bone age was approximately 16–19 years. Among the significant findings were: increased thickness of the skull bones, fusion of the base of the skull, complete ossification of the scapulas, fusion of all the epiphyses above the elbows, almost complete fusion of all the vertebral elements in the dorsal and lumbar spine, calcification of the costal cartilages, the appearance of the secondary epiphyses of the iliac crest and ischial tuberosities and complete fusion of the heads and trochanters of the femora. There was lumbarization of the first sacral segment in Eileen but not in Maureen. The sella turcica was normal in size and contour. There was no evidence of adrenal tumor or enlargement by intravenous urogram and pre-sacral air study. The right adrenal was never clearly visualized in either twin, however, on two attempts.

Biopsy specimens of skin from the flexor surfaces of the forearms of each subject were obtained.[3] The sex chromatin pattern was female in 70 per cent of the nuclei. The sections were reviewed by Dr. Murray L. Barr (Professor of Microscopic Anatomy at the University of Western Ontario, Faculty of Medicine), who confirmed the dominant female pattern.

Blood grouping studies[4] are shown in Table 2. The twins inherited the mother's blood types for the complete list of anti-sera. There was an approximate 98 per cent probability that the twins were identical, based on their blood grouping characteristics.

On June 14, 1955, a hydrocortisone suppression test (8) was performed. A dose of 100 mg. of hydrocortisone in 5 per cent dextrose-distilled water was infusedi ntravenously over a 24-hour period. Pre-infusion urinary 17-ketosteroids were 20.8 mg. (Maureen) and 48.6 mg. per twenty four hours (Eileen). During the infusion the values were 6.0 mg. and 6.2 mg.; the following day, 6.9 mg. and 8.4 mg.; and on the second post-infusion day, 27.5 mg. and 36.3 mg. per twenty-four hours. Urinary pregnanetriol values before hydrocortisone were 70 and 64 mg. per twenty-four hours, respectively, and 56 and 42 mg. during the infusion. On June 17, a dose of 25 mg. of ACTH in 5 per cent dextrose-saline solution was administered intravenously over an eight-hour period. Urinary 17-ketosteroids rose to 76.4

[3] By Dr. Harry Roitman, Resident in Obstetrics and Gynecology, Albert Einstein Medical Center (Southern Division).

[4] Mr. A. Kahn, Serologist, Albert Einstein Medical Center (Southern Division).

mg. and 82.6 mg., respectively; on June 18 they were 50.1 and 73.0 mg.; and on June 19, 1955, they were 27.1 and 44.0 mg. per twenty-four hours. Urinary pregnanetriol rose from base-line values of 65 mg. and 58 mg. per twenty-four hours to 87 mg. and 76 mg. after administration of ACTH. The results of the hydrocortisone and ACTH tests in Maureen are illustrated in Figure 3. The values for Eileen were similar and are therefore not included.

TABLE 2. BLOOD GROUPING STUDIES

Anti-Sera	Mr. McG.	Mrs. McG.	Eileen	Maureen
A	+	—	—*	—*
B	—	—	—	—
C	—	—	—	—
D	—	+	+*	+*
E	—	—	—	—
c	—	+	+*	+*
e	+	+	+	+
M	+	+	+	+
N	+	+	+	+
P	—	—	—	—
K (Kell)	+	—	—*	—*
Le^a (Lewis)	—	+	+*	+*
Fy^a (Duffy)	—	+	+*	+*
s (Sanger)	+	—	—	—
C^w (Variant)	—	—	—	—
D^n (Variant)	—	0	0	0
JK^a (Kidd)	—	+	+*	+*

* Significant points of similarity between the twins.

CLINICAL COURSE

Therapy was started on June 24, 1955, with 50 mg. of cortisone acetate given intramuscularly every twelve hours. Figures 3 and 4 illustrate the urinary 17-ketosteroid responses of Maureen to the various therapies that were used. The values obtained for Eileen were similar. The twins were discharged from the hospital on July 1, 1955. The initial therapeutic response to the injection of 100 mg. of cortisone acetate was not consistently satisfactory, using the urinary excretion of 17-ketosteroids as a criterion. Values above 8 mg. per twenty-four hours, the desired maximum level, were frequent. Acne subsided rapidly, however, and breast budding appeared within two months and progressed thereafter. There was a slight elevation of the pitch of their voices and hair growth on the face, arms and legs appeared lighter in color and softer in texture. Because Segaloff *et al.* (9) had reported the successful suppression of adrenal function in 5 pa-

tients with adrenal hyperplasia, using infrequent injections of a saline suspension of hydrocortisone, this material[5] was given a trial commencing July 28, 1955. In Figure 3 the frequency of injections, the dose used and the laboratory response are illustrated. The suppression of 17-ketosteroids was at first satisfactory, but hypertension, moon-face, edema, striae of the thighs and hypernatremia occurred, necessitating a reduction in the frequency of injections and the dose. In November 1955 the adrenals escaped from the suppressive effects of this therapy despite the appearance of Cushingoid phenomena.

[5] Hydrocortisone, saline suspension, Lot #55R992 (with 0.9% benzyl alcohol as a preservative), Merck & Co.

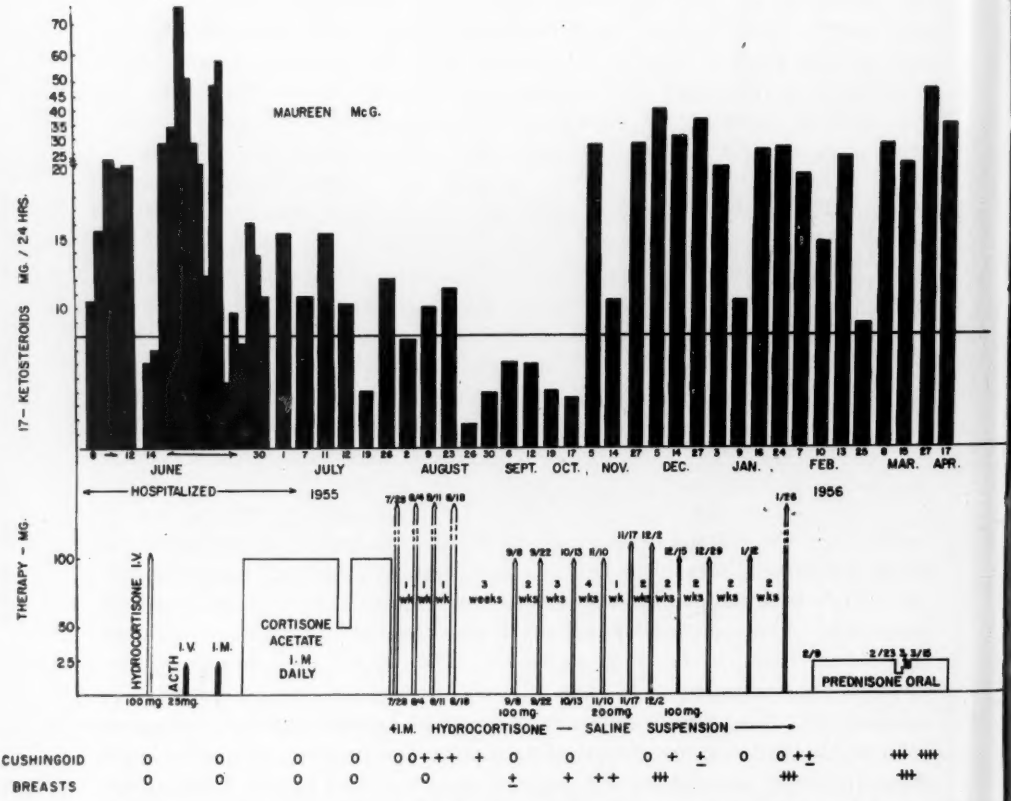

FIG. 3. The urinary 17-ketosteroid response to therapy in Maureen McG. The horizontal line through the black bars represents the maximum desirable 17-ketosteroid level in children with congenital adrenal hyperplasia (8 mg.). Note change in scale for 17-ketosteroids above 20 mg./24 hrs.

In February 1956 the use of hydrocortisone was abandoned and oral prednisone, in fractional doses varying from 15 to 25 mg. (total) daily, was substituted. Again lack of suppression coupled with marked Cushingoid side-effects was observed. The patients were readmitted to the hospital on May 7, 1956, for further study because of the possibility that an adrenal tumor might have been overlooked. The feasibility of clitorectomy at this time was another reason for hospitalization.

The twins were now 60 inches in height; weight 104¾ pounds (Maureen) and 100 pounds (Eileen); and blood pressure 170/118 mm. Hg (Maureen) and 140/82 (Eileen). There was marked facial edema, puffiness of the hands and feet, some facial acne, and many salmon-pink striae over the upper anterior portions of the thighs. Eileen was found to have a right basal pneumonitis which was rapidly controlled with antibiotic therapy.

X-ray examination of the skeleton showed almost complete fusion of the epiphyses of the distal ends of the femora, tibiae and fibulae, whereas previously these epiphyseal lines had been well visualized. Results of routine blood and urine examinations were normal. The serum sodium, potassium and chloride levels for Eileen were 138.5, 5.0 and 93 m.Eq./L. and for Maureen, 140.4 5.2 and 88 m.Eq./L. The urinary 17-ketosteroid values and therapy during the second hospitalization for Maureen are illustrated in Figure 4.

Cortisone was administered intramuscularly in dosages of 25 to 50 mg. per day. The urinary 17-ketosteroids were reduced from an average daily level of approximately 30 mg. during the months immediately preceding hospitalization to an average daily level of approximately 13 mg. during the last four weeks of their second hospital stay. On June 6, oral administration of 9α-fluorohydrocortisone was started (5 mg. per day fractional doses), in the hope of eliminating injections. Though there was fair suppression of urinary 17-ketosteroids, Cushingoid effects were prominent. Fluorohydrocortisone was stopped and intramuscular cortisone readministered. The psychiatric department believed that more intensive psychologic preparation was necessary prior to clitorectomy and suggested that this procedure be indefinitely postponed. The twins were discharged from the hospital on June 16, 1956, while receiving 50 mg. of cortisone intramuscularly daily.

Following discharge from the hospital Eileen was given prednisone intramuscularly, starting with 15 mg. per day; a satisfactory maintenance dose was found to be 8 mg. every other day. Urinary 17-ketosteroids remained consistently below 8 mg. per twenty-four hours during this regimen, except for a single value of 15.6 mg. on October 9, 1956. Maureen showed similar good results while receiving 25 mg. of cortisone intramuscularly every other day. On March 26, 1957, the two medications were inter-

changed, Eileen receiving cortisone and Maureen prednisone. The urinary 17-ketosteroid values remained below 4 mg. per twenty-four hours. Pregnanetriol could not be recovered from the urine when this study was repeated in April 1957. In June and July 1958 a trial of 6-methylprednisolone in an oral dosage of 4 mg. twice daily for fifty days was associated with a rise of urinary 17-ketosteroids from less than 4.0 mg. to 11–15 mg. per twenty-four hours, and the appearance of mild facial acne.

Since discharge of the twins from the hospital in June 1956, acne has disappeared completely, hair on the upper lip, sides of the face and on the extremities has become much less prominent, the voices have assumed a more feminine pitch, and the striae on the thighs have faded. Breast

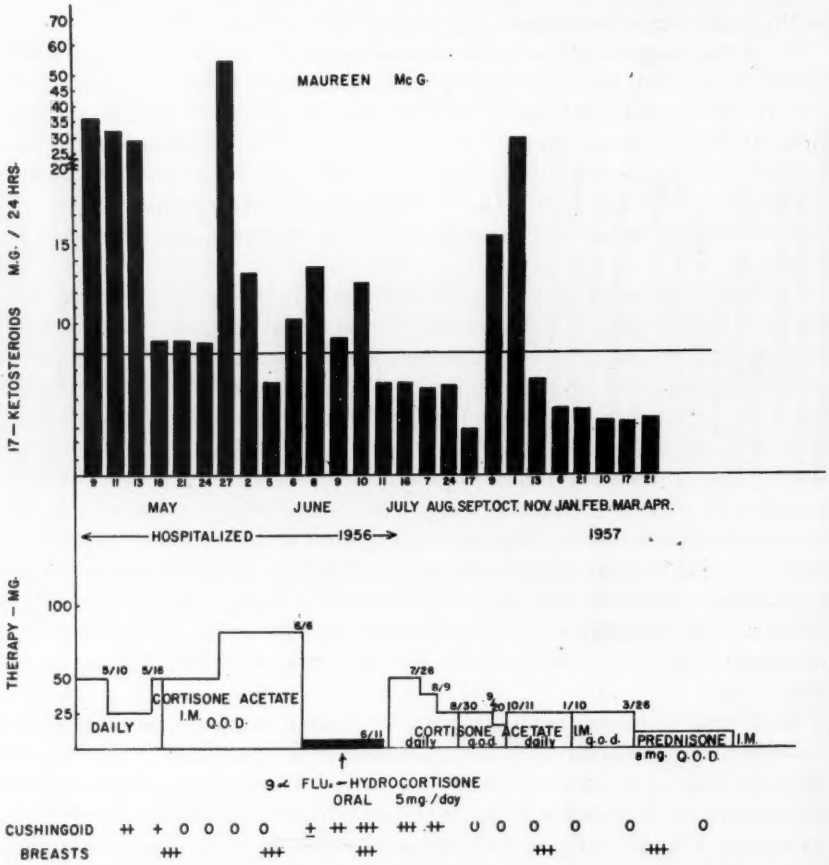

FIG. 4. The urinary 17-kestosteroid response to therapy in Maureen McG. during and after the second hospitalization. Note change in scale for 17-ketosteroids above 20 mg./24 hrs.

development is that of normal adolescence. Menarche appeared in August 1958. Their ability to withstand stress has been satisfactory and their physical strength, endurance and school performance have been excellent.

DISCUSSION

The occurrence of congenital virilizing adrenal hyperplasia in identical twins lends support to the concept of a genetic origin. The rarity of this syndrome in identical twins is not surprising. The incidence in Maryland (1), the only available data to our knowledge, has been estimated to be 1 per 67,000 births. According to Hurlock (10), twinning appears once in 85 births and approximately 25 per cent are identical, *i.e.*, the rate for identical twins is 1 per 340 births. One would therefore expect the incidence of congenital virilizing adrenal hyperplasia to be 1 per 23 million births. Failure to arrive at a diagnosis, as in miscarriage, mild states, the difficulty of recognition in males, or early death from adrenal insufficiency would tend further to reduce the apparent incidence compared with the true incidence. There is also the more remote possibility that some cases may not be reported. One would expect, therefore, that recognized and reported examples would occur even less often than 1 per 23 million. The national birth rate in the past twenty years has averaged about 3 million per year, so that this disease would be expected to occur in identical twins approximately once in seven and a half years. Modern diagnostic tools for the recognition of congenital virilizing adrenal hyperplasia have been available only in the past two decades, and real interest in the condition has been shown only in the past few years. It is not surprising that only 1 previous authenticated example has been reported.

In our 2 patients considerable difficulty was encountered initially in attaining good suppression of adrenal androgen production without causing undesirable Cushingoid effects. Infrequent intramuscular injections of a saline suspension of hydrocortisone did not achieve adequate suppression without causing Cushingoid phenomena and was abandoned. Since his first publication, Segaloff[6] has found this therapy very successful in a larger group of patients. Our failure to duplicate his experience may perhaps be explained by the fact that we injected the material undiluted rather than in 10 cc. of normal saline as advocated by him. Irregularly absorbed depots of steroid may have prevented continuous pituitary suppression, and may have occasionally flooded the blood stream with corticoids. Oral therapy with prednisone, 9α-fluorohydrocortisone or 6-methylprednisolone failed to suppress the urinary 17-ketosteroids adequately, but (except for 6-methylprednisolone) did produce severe Cushingoid effects. This has been the experience of other workers in occasional cases. Conn and associates

[6] Segaloff, A.: Personal communication, 1957.

(11) have suggested that oral administration leads to a greater hepatic conversion of corticosteroid compounds to 17-ketosteroids; thus larger oral doses may be required to lower the patient's daily 17-ketosteroid excretion to the desired level (8 mg. or less).

A successful interchangeable regimen was finally achieved in these twins, consisting of either 25 mg. of cortisone or 8 mg. of prednisone injected intramuscularly every other day.

Acknowledgments

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DETERMINATION OF PROGESTERONE IN HUMAN BLOOD PLASMA*

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ABSTRACT

A procedure for the determination of progesterone in plasma is described. By means of a reaction with sulfuric acid-ethanol, 0.5 μg. of progesterone may be determined. The preliminary application of this method to the estimation of progesterone in 10 ml. of pregnancy plasma and 15–20 ml. of normal plasma indicates that levels of plasma progesterone can be studied.

MOST present methods for the determination of progesterone in biologic material (1–3) depend upon ultraviolet absorption of the free steroid or its derivatives (1–6). Such procedures necessitate elaborate purification of extracted material, and an amount of progesterone approximating 5 μg. is required for adequate ultraviolet resolution. Since the plasma levels of progesterone during pregnancy may be as low as 4 μg. per 100 ml. (2), large volumes of plasma are needed for a single determination.

In this communication a modification of the method of Zander and Simmer (1) is reported. This procedure permits determination of 0.5 μg. of progesterone in 20 ml. of plasma with fair accuracy.

EXPERIMENTAL

A. *Precipitation of protein*

To each sample of blood plasma is added 1000 cpm of radioactive progesterone.[1] The plasma is thoroughly shaken for fifteen minutes with three times its volume of absolute ethanol (EtOH) in a centrifuge tube or bottle. After centrifugation the extract is transferred into a second centrifuge tube or bottle and the precipitate is extracted twice with 0.5 volume of absolute EtOH each time. The combined EtOH extracts are evaporated to dryness *in vacuo* at 40° C.

B. *Removal of lipid material*

The residue of the EtOH extract is dissolved in 10 ml. of 70 per cent methanol (MeOH) and kept at −15° C. for fifteen hours before it is centrifuged for fifteen minutes

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[1] 1 μg. of this material contains 66,000 cpm of radioactivity.

at 2000 rpm in a refrigerated centrifuge. The supernatant liquid is carefully removed and the remaining precipitate is washed once with 2 ml. of ice-cold 70 per cent MeOH.

C. *Extraction of progesterone*

After addition of 15 ml. of water to the 70 per cent MeOH solution, the material is extracted three times with 15 ml. of hexane each time. The combined hexane extracts are evaporated *in vacuo*.

D. *Concentration of the extract on paper strips*

The dry residue is applied on a 4 cm. ×12 cm. Whatman No. 1 paper strip (7) using three times 0.2 ml. of MeOH-chloroform (1:1 v/v). In a similar fashion a 50-μg. sample of deoxycorticosterone is applied to the same paper strip as a reference compound, and the strip is submitted to ascending chromatography with MeOH-benzene (1:9 v/v) (7). Within three to four hours the free steroids are concentrated on the tip of the strip.

E. *Decreasing paper chromatography*

The tip of the concentration strip containing the extracts is cut off and inserted into corresponding cuts on impregnated paper strips. For impregnation of the Whatman No. 1 paper, a mixture of propylene-glycol and MeOH (1:1 v/v) is used. The chromatogram is developed with methylcyclohexane (8). With every chromatogram, a 50-μg. aliquot of progesterone is run alongside on one strip as reference standard. The R_{DOC} value (relative mobility of progesterone, referred to that of deoxycorticosterone (DOC)) lies between 4.8 and 5.2 in this system. After ten to twelve hours, the paper chromatograms are removed and thoroughly dried. A blank strip containing no steroids is taken through the total procedure of paper chromatography.

F. *Elution of progesterone*

The spots of progesterone and deoxycorticosterone on the standard strip are located by the ultraviolet scanning technique and the R_{DOC} value of progesterone is determined. The area of the sample strip exhibiting the same R_{DOC} value as that of the standard strip is cut out and extracted three times with 2 ml. of MeOH each time. Likewise, 2 pieces of paper from a blank strip corresponding in size and location to that of the sample strip are eluted, and a 10-μg. sample of progesterone is added to one of these eluates. The 3 eluates are then evaporated to dryness *in vacuo*.

G. *Purification of paper eluates*

The dry residues are redissolved in 10 ml. of benzene and extracted once with 10 ml. of 0.1 *N* sulfuric acid and twice with 10 ml. of water each time. The benzene solution is evaporated to dryness *in vacuo*.

H. *Quantitative determination of progesterone*

The unknown plasma sample is dissolved in 1 ml. of EtOH, and 0.1 ml. of this solution is plated and counted by means of a windowless counter. The remaining EtOH solution of the unknown sample is evaporated to dryness. The dry residues of the 3 extracts—sample, standard and paper blank—are dissolved in 1.2 ml. of a reagent prepared from 1 volume of 80 per cent EtOH (obtained from 95 per cent EtOH freshly distilled over Ag_2O *in vacuo*) and 2 volumes of concentrated sulfuric acid (analytical grade). The reaction mixture is warmed for fifteen minutes at 60° C. and subsequently cooled. The solution is transferred into a 1.5-ml. cuvette (Pyrocell) and the absorption spectrum is registered in a Beckman DK-2 recording spectrophotometer between 250

and 340 mμ, or in a Beckman DU spectrophotometer at 260, 290 and 320 mμ, using a hydrogen lamp as light source and the paper blank as reference. The use of a 0.5-ml. cuvette in the Beckman DU spectrophotometer will increase the sensitivity of the reaction, since less reagent is needed.

The maximum absorbance (Abs.) at 290 mμ is corrected using a formula similar to that of Allen (9)[2]:

$$\text{Corrected Abs.}_{.290} = \text{Abs.}_{.290} - \frac{(\text{Abs.}_{.260} + \text{Abs.}_{.320})}{2}$$

The amount of progesterone in the unknown plasma sample is then calculated from the recovery of labeled and nonlabeled progesterone taken through the entire procedure.

Heparin is used as an anticoagulant for whole blood. If the plasma samples cannot be processed immediately, they should be kept frozen until they are submitted to the procedure.

RESULTS

With the color reaction described, 0.5 μg. of synthetic progesterone can be measured with adequate accuracy (Fig. 1). From Table 1 it is apparent

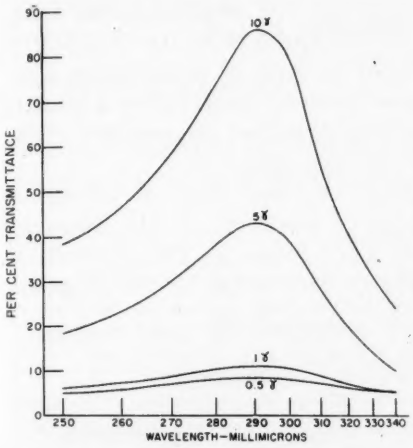

FIG. 1. Absorption curves of 0.5, 1.0, 5.0 and 10.0 μg. of standard progesterone in 1.2 ml. of reagent.

Corrected absorbance (9) at 290 mμ:

0.5 μg. = 0.021 = 0.0420/μg.
1.0 μg. = 0.041 = 0.0410/μg.
5.0 μg. = 0.216 = 0.0432/μg.
10.0 μg. = 0.422 = 0.0422/μg.

0.0421 ± 0.008. (mean absorbance and one standard deviation)

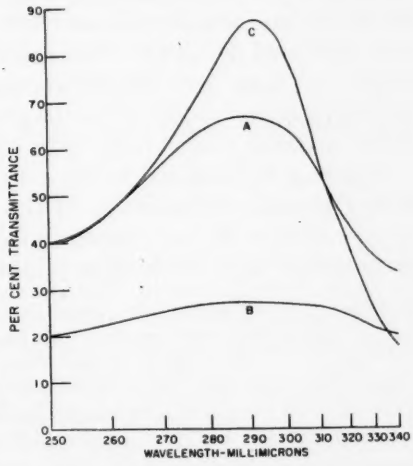

FIG. 2. Absorption curves of progesterone in 1.2 ml. of reagent.

A) 25 ml. of pregnancy plasma (thirty-seventh week).
B) 20 ml. of normal plasma (seventh day after menstruation).
C) 10 μg. of standard progesterone.

[2] Human male plasma processed by this method exhibited a linear absorption curve from 260–320 mμ.

TABLE 1. CORRECTED ABSORBANCE (9) AT 290 Mμ OF 10 μG. OF PROGESTERONE AT DIFFERENT TIME INTERVALS AFTER ADDITION OF 1.2 ML. OF THE EtOH-SULFURIC ACID REAGENT

Minutes after addition of reagent	Temperature (Centigrade)	Corrected absorbance at 290 mμ
1	60°	0.389
5	60°	0.414
10	60°	0.431
15	60°	0.438
20	60°	0.437
25	60°	0.438
30	60°	0.435
60	25°	0.436
120	25°	0.432
180	25°	0.425

that the color produced with the sulfuric acid reagent is stable for at least two hours. The corrected mean absorbance for 1 μg. of progesterone using different concentrations of the hormone was found to be 0.0478 ± 0.0014 (one standard deviation[3]). When different amounts of progesterone were added to a 6 per cent protein solution or to blood plasma from a normal male subject and submitted to the procedure described, the recovery of the added material ranged from 78 to 101 per cent (Table 2).

Applying this method to the estimation of progesterone in plasma, a higher plasma concentration of this steroid was found in the pregnant woman than in the nonpregnant (Table 3 and Figure 2). Plasma levels of progesterone agree with those published by Zander (2) for the pregnant

TABLE 2. RECOVERY OF DIFFERENT AMOUNTS OF PROGESTERONE ADDED TO 10 ML. OF A 6 PER CENT SOLUTION OF HUMAN SERUM PROTEINS IN WATER, OR TO 10 ML. OF PLASMA FROM A NORMAL MALE SUBJECT

	Progesterone added (μg.)	Progesterone recovered (μg.)	Recovery (per cent)
6 per cent solution of human serum proteins in water	0.5	0.41	82
	1.0	0.85	85
	5.0	3.97	78
	10.0	8.76	87
10 ml. of plasma from a normal male subject	2.0	2.03	101
	3.0	2.91	97
	4.0	3.93	98
	10.0	8.63	86

[3] N = 31 observations. The mean corrected absorbance reported is calculated per μg. of progesterone per ml. of reagent.

TABLE 3. PLASMA LEVELS OF PROGESTERONE IN NORMAL AND PREGNANT WOMEN

Subject	Plasma vol. extracted (ml.)	Condition	Plasma progesterone (μg./100 ml.)
P.F.	20	Seventh day after menstruation	5.0
D.L.	20	Ninth day after menstruation	3.6
A.W.	20	Twelfth day after menstruation	2.5
L.G.	18	Fifteenth day after menstruation	3.2
J.B.	20	Twenty-first day after menstruation	4.7
L.G.	17	Ninth week of pregnancy	11.9
P.H.	16	Thirteenth week of pregnancy	16.6
A.K.	10	Thirty-fifth week of pregnancy	18.7*
			20.7
J.H.	10	Thirty-seventh week of pregnancy	19.2*
			17.3
R.O.	10	Thirty-seventh week of pregnancy	26.6*
			22.9
T.L.	13	Thirty-seventh week of pregnancy	22.0
P.B.	10	Forty-first week of pregnancy	44.9

* Sample run in duplicate.

woman. Circulating levels of plasma progesterone have not hitherto been reported for the nonpregnant woman.

DISCUSSION

As shown by Zaffaroni *et al.* (10), the absorption spectrum of progesterone in concentrated sulfuric acid exhibits a distinct absorption maximum at 290 mμ. Since, however, contaminating substances present in biologic material react with concentrated sulfuric acid to produce an unspecific color, the concentration of the reacting sulfuric acid was decreased to approximately 66 per cent. This concentration of sulfuric acid is only slightly higher than that used in the Porter-Silber reaction (11) for 17-hydroxycorticosteroids which is widely used in clinical laboratories and yields reproducible results (12). One would therefore infer that the 66 per cent sulfuric acid concentration used in the present method should not introduce hazardous background interference. The formation of chromogen in a mixture of progesterone and sulfuric acid is unspecific, and separation of progesterone from other steroids in the plasma is necessary. Adequate resolution was obtained, however, with the propylene glycol-methylcyclohexane system of Savard (8).

The method described allows the detection of as little as 0.5 μg. of progesterone in biologic fluids. Thus the circulating plasma concentration of progesterone can be determined in the nonpregnant woman using 15–20

ml. of plasma (Fig. 2). Hence, experiments pertinent to plasma progesterone can be conducted without having to draw an excessive amount of blood.

For women during pregnancy, the levels of plasma progesterone detected by the present method agree with those observed by Zander (2). We have observed low concentrations of plasma progesterone in the nonpregnant woman—apparently too low to be measured adequately by the original Zander and Simmer technique (1). By the present color reaction, these plasma progesterone concentrations yield a chromogen spectrum similar to that of synthetic progesterone. In addition to chromatographic behavior, this spectrum strongly indicates that the measured progesterone in normal plasma actually is this steroid hormone.

Acknowledgments

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PLASMA CORTISOL LEVELS IN MAN FOLLOWING ORAL AND INTRAVENOUS ADMINISTRATION OF CORTISOL*

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ABSTRACT

Plasma cortisol levels were determined at intervals following the administration of cortisol. After oral administration, the mean cortisol concentration reached a peak at one hour which was significantly higher in females. After intravenous administration, mean values were also higher in females than males, but this difference was not significant. Possible explanations for these findings are discussed.

INTRODUCTION

SEVERAL investigators (1–7), using methods of differing specificity, have measured blood levels of cortisol in normal subjects following administration of this steroid. In the present investigation the response to oral and intravenous cortisol has been studied in male and female subjects, using a specific method for cortisol (8) which incorporates a correction by isotope dilution for losses during analysis.

METHODS

The subjects chosen were convalescent hospital in-patients. Patients with cardiac, hepatic, renal or endocrine dysfunction were excluded. The composition of the male and female groups in respect of age and body weight is shown in Table 1. In the majority of instances only 1 study was made on each patient, in order to minimize inconvenience to the patient. A larger dose of cortisol was used for the oral than for the intravenous test, with the object of establishing comparable blood levels.

Oral cortisol (21 subjects)

Administration was started at 9 A.M. after an overnight fast. A 50-mg. dose of cortisol (Ef-Cortelan, Glaxo) in 10 ml. of 50 per cent ethanol was diluted to 100 ml. with water flavored with orange, yielding a final alcohol concentration of 5 per cent. Blood samples were taken before, and at intervals of one, two and four hours after oral administration

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of the dose. The subject was allowed a cup of tea and a slice of toast at 10 A.M., and lunch was taken at the usual time of 12 noon.

Intravenous cortisol (39 subjects)

Administration was also started at 9 A.M. but the subjects were not fasting. A 25-mg. dose of cortisol in 5 ml. of 50 per cent ethanol was added to 50 ml. of sterile physiologic sodium chloride, yielding a final alcohol concentration of 4.5 per cent. The solution was injected slowly intravenously, taking about three minutes. In most cases blood was drawn at fifteen minutes, one hour, two and a half hours, and six hours after the injection. In 10 of the 39 subjects blood was drawn at fifteen minutes only.

TABLE 1. AGES AND BODY WEIGHTS OF SUBJECTS RECEIVING CORTISOL

	Oral cortisol		Intravenous cortisol	
	Males	Females	Males	Females
Age in years (mean & range)	50 (21–69)	50 (25–72)	47 (22–65)	51 (17–74)
Weight in pounds (mean & range)	139 (93–179)	130 (115–156)	140 (119–158)	125 (93–191)
No. of subjects	10	11	21	18

Determination of cortisol in plasma

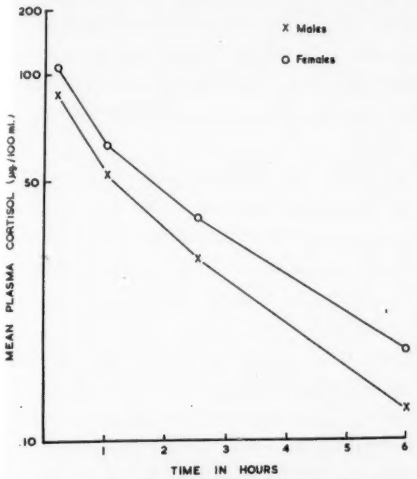
Immediately after withdrawal the blood was transferred to bottles containing heparin. Plasma was separated by centrifugation within a few minutes. Cortisol was estimated using the method of Bondy *et al.* (8) for extraction and paper chromatography, and the fluorometric method of Sweat (9) for the estimation of cortisol in the paper eluates.

RESULTS

Before oral administration (Fig. 1) the mean fasting plasma cortisol concentration was 9.9 μg. per 100 ml. in males (range, 0–28.7 μg.) and 12.4 μg. per 100 ml. in females (range, 0–32 μg.). The means did not differ significantly ($P > 0.5$). One hour after the oral dose of cortisol the mean plasma cortisol level reached a peak of 43 μg. per 100 ml. in males and 88 μg. per 100 ml. in females. This difference is highly significant ($P < 0.01$). Subsequently plasma cortisol levels fell, but at four hours the mean value in both males and females still exceeded the mean fasting concentration.

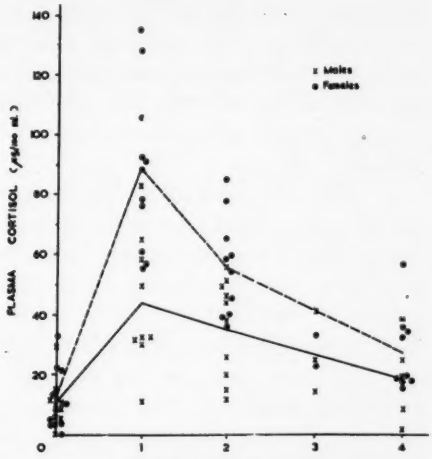
After intravenous administration (Fig. 2) the mean plasma cortisol concentrations at fifteen minutes, one hour, two and a half hours and six hours were higher in females than in males, but at no time was the difference significant. The combined probability of the aggregate of the 4 differences (10) was also not significant ($\chi^2 = 13.3772$, $n = 8$, $0.1 > P > 0.05$).

In addition to the ultraviolet absorbing spot due to cortisol, we have frequently observed 3 other ultraviolet absorbing spots on paper chromatograms of plasma extracts. One of these (Rf = 0.5–0.6) has been shown to be due to caffeine or a derivative (11). The others, more polar than cortisol, have not yet been identified, but we have no evidence that they are steroids. Bush (12) has also reported the presence of 3 ultraviolet absorbing spots on chromatograms of plasma extracts. However it is not possible to say whether or not they correspond with the spots just described, because we used chloroform instead of ethyl acetate for the extracting solvent, and toluene-75% methanol instead of bezene-55% methanol for the paper chromatographic separation.



	MEAN PLASMA CORTISOL (µg/100 ml.) ±S.E.			
	15 MIN.	1 HOUR	2½ HOURS	6 HOURS
MALES	87 ±6·8 (21)	55 ±6·6 (14)	32 ±3·3 (13)	11·8 ±2·4 (12)
FEMALES	106 ±7·2 (18)	64 ±6·5 (14)	41 ±5·4 (14)	17·5 ±5·5 (13)
P VALUES	>0·05	>0·3	>0·1	>0·3

FIG. 1. Oral cortisol tolerance curves.



	MEAN PLASMA CORTISOL (µg/100 ml.) ±S.E.			
	0 HOURS	1 HOUR	2 HOURS	4 HOURS
MALES	9·9 ±2·5 (10)	43 ±7·3 (9)	35 ±5·1 (10)	18 ±6·2 (5)
FEMALES	12·4 ±2·8 (11)	88 ±8·1 (11)	55 ±5·2 (10)	27 ±3·8 (9)
P VALUES	>0·5	<0·01	<0·02	>0·2

FIG. 2. Intravenous cortisol tolerance curves.

No ultraviolet absorbing derivatives of cortisol were consistently found following either oral or intravenous administration.

DISCUSSION

The difference in plasma levels between males and females following oral administration of cortisol could theoretically be attributed to:

1. Differences in absorption of cortisol from the gastro-intestinal tract. This possibility is supported by the very small sex difference observed after intravenous administration. On the other hand, all the administered cortisol would be carried by way of the portal vein to the liver following absorption, whereas the intravenously administered material would be distributed throughout the systemic circulation after passing through the heart and lungs. Hence one might expect that a hepatic effect on cortisol disposal would also be more pronounced after oral than after intravenous administration. The dominant role played by the liver in cortisol metabolism has been shown both by the decreased rate of removal of intravenous cortisol in patients with liver disease (13), and by the large arterial-hepatic venous difference observed following intravenous infusion of the steroid (14).

2. Different volumes of distribution following absorption. The similarity of the intravenous curves for males and females, in particular the relatively minor (and statistically insignificant) difference in the mean values at fifteen minutes, makes this explanation unlikely. (The mean initial volume of distribution derived from the curves in Figure 2 is 22 liters—rather more than the extracellular fluid volume.)

3. Different rates of degradation, tissue utilization or renal excretion following absorption. The possible effects of hepatic disposal have already been mentioned. Inspection of the curves obtained from the intravenous test data by plotting the mean plasma cortisol concentration on a semi-logarithmic scale against time shows that the final rates of removal are very similar in the two sexes, the half-time of this final process being approximately 2.4 hours, in agreement with the findings of other workers (4, 6, 7).

4. Different degrees of suppression of endogenous cortisol production. The magnitude of the sex difference in the oral test makes this improbable, and in addition one might have expected that the effect of intravenous cortisol would be more marked than that of oral cortisol.

In summary, the necessarily circumstantial evidence points to either decreased alimentary absorption or increased hepatic disposal of orally administered cortisol in males.

Following oral administration of cortisol the maximum plasma concentration occurred at one hour, in accordance with the findings of Nelson *et al.* (15). This suggests that absorption takes place either in the stomach or in the upper part of the small intestine. However, a possible effect of the small quantity of alcohol present on the site of absorption cannot be excluded.

No obvious correlation was noted in either the oral or intravenous tolerance curve between age and plasma cortisol level, or between body weight and plasma cortisol level.

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HYPOPARATHYROIDISM: REPORT OF A CASE SUCCESSFULLY TREATED WITH INTRAVENOUS VITAMIN D_2

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ABSTRACT

A case of surgical hypoparathyroidism with unusual resistance to ordinary methods of treatment is reported. The successful use of a new intravenous preparation of vitamin D_2 is described. A brief discussion is included concerning the current aspects of the use of vitamin D preparations in hypoparathyroidism and the possible cause of resistance in this patient.

THE treatment of hypoparathyroidism is based on the use of vitamin D_2 (calciferol) or AT10 (dihydrotachysterol) given orally to cause increased absorption of calcium from the intestine into the blood, thereby raising the level of serum calcium. Agents having a supposed phosphaturic effect, such as probenecid (Benemid) have proved to be of questionable benefit (1, 2). The use of parathyroid hormone is not recommended because of the fairly rapid development of antibodies to the preparation now commercially available; however, this is poorly documented in the literature and very little factual information is available about the use of parathyroid hormone in human subjects.

This paper will deal with a case of surgical hypoparathyroidism which was unusual in that resistance to extraordinarily large doses of calciferol and AT10 was demonstrated. Only with a new preparation of intravenous vitamin D_2 was successful treatment effected.

CASE REPORT

J.H. (*PBBH #5k909*) was a 17-year-old schoolgirl who was admitted to the Peter Bent Brigham Hospital for the first time in January 1957, for removal of a "cold" nodule of her thyroid gland. Her past history was noncontributory. Results of physical examination were negative except for a large nodule in the left lobe of the thyroid. A radioactive iodine uptake test showed the nodule to be nontoxic. A total thyroidectomy was performed in January 1957, at which time the diagnosis of papillary adenocar-

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cinoma with metastases to regional lymph nodes was established. At operation, 2 parathyroid glands were seen and carefully left in place. Postoperatively, however, severe tetany developed, with laryngeal stridor, paresthesias and muscle spasm; the serum calcium level fell to 3.0 mEq. per liter. Careful scrutiny of all tissue removed at operation did not reveal any parathyroid tissue. The patient was given varying doses of calciferol and AT10, a high-calcium low-phosphorus diet, and 3 grains of desiccated thyroid daily, but the tetany remained unabated (Fig. 1). At this time the renal tubular reabsorption of phosphate was 97 per cent.

In April 1957, November 1957, and again in January 1958 this patient received a transplantation of parathyroid tissue. Two of these grafts were in diffusion chambers (3, 4), and 1 was a vascularized fetal thyro-parathyroid graft (5). One of the diffusion-chamber grafts was taken from a patient with primary parathyroid hyperplasia, and the other was taken from the patient's cousin. The fetal graft was anoxic for at least six hours before blood flow was re-established. Subsequent studies of the function of these homotransplants will be reported separately.

Over the next year, increasing dosages of calciferol and/or AT10 were tried, reaching levels of 5,000,000 units[1] daily, without any apparent effect.

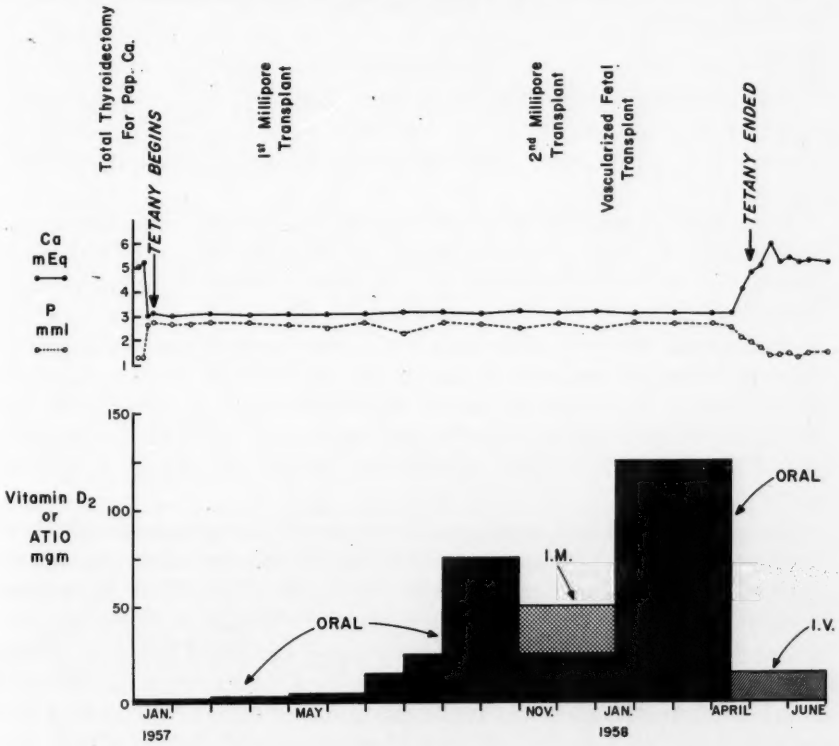

FIG. 1. Clinical course correlated with levels of vitamin D_2 therapy.

[1] One milligram of vitamin D_2 or D_3 or of AT10 equals 40,000 units.

In November 1957 it was thought that she might be suffering from gastro-intestinal malabsorption. It was demonstrated that blood levels of vitamin A and carotene were normal, but that butterfat absorption was abnormal, as was xylose absorption. X-ray studies of the gastro-intestinal tract showed no abnormalities. A trial of pancreatin therapy was without benefit, as were courses of Benemid and cortisone. Then calciferol in dosages up to 1,000,000 units per week intramuscularly was tried for several weeks, but had no apparent effect.

In February 1958 the patient had convulsions, and was treated with Dilantin. X-ray examination of the skull did not show any calcification of the basal ganglia. In March 1958 the results of an Ellsworth-Howard test were strongly positive.

In April 1958 an aqueous intravenous preparation of vitamin D_2 called Vi-DeHydrosol[2] was obtained. This was given in a dosage of 15 mg. (600,000 units) intravenously once weekly. Within three weeks after initiating this therapy the serum calcium level had risen to within normal limits and the serum phosphorus level had fallen. The tubular reabsorption of phosphate was 94 per cent, and tetany was no longer present for the first time in fifteen months. The patient was subjectively and objectively normal and no Chvostek and Trousseau signs could be elicited. At the present time the serum calcium level ranges from 4.8 to 5.3 mEq. per liter, and the phosphorus level from 1.3 to 1.7 mM. per liter.

DISCUSSION

The usual causes for resistance to oral vitamin D are: 1) sprue or the malabsorption syndrome, 2) an inherited condition, as in "resistant rickets," 3) hypometabolic states, as in myxedema, and 4) acquired resistance (6).

The adult requirement of vitamin D has never been established, but apparently it is required in some form, as otherwise pure osteomalacia is reported to occur (7). The amount of vitamin D required for the prevention of osteomalacia is probably very small—about 40 to 400 units or 1 to 10 micrograms daily (8). This amount is approximately the equivalent of that received from exposure to bright sunlight for half an hour daily. In uncomplicated hypoparathyroidism the requirement of vitamin D for maintenance of normal levels of serum calcium is much higher, roughly 50,000 to 300,000 units daily coupled with an adequate intake of calcium in the diet.

Our patient exhibited an unusual resistance to the ordinary methods of treatment for the hypoparathyroid state. Tetany persisted for fifteen months in spite of very large oral doses of vitamin D_2 or AT10. Myxedema was not present at any time in the course of the disease, and she had been taking 3 grains of desiccated thyroid daily since the initial operation. There was no history of steatorrhea, but the results of laboratory tests indicated malabsorption. Whether this faulty gastro-intestinal absorption was secondary to the low blood calcium level has not as yet been established, but studies are in progress concerning this point.

[2] Supplied by Dr. A. Wander & Co., S. A., Berne, Switzerland.

The response to intravenous aqueous vitamin D_2 was remarkable. Within two to three weeks after starting the injections the serum calcium concentration was normal and has been maintained at this level with 15 mg. (600,000 units) intravenously per week. Actually, except for their speed of action in raising the level of blood calcium, very little is known about the comparative properties of vitamin D_2, vitamin D_3 and AT10, including their absorption, fate in the body and excretion. To date, the data have been inadequate, in that careful balance studies have not been undertaken comparing the various preparations (9, 10). There is little doubt, however, that in this patient intravenous vitamin D_2 exerted its effect promptly and that the patient has been maintained in a euparathyroid state successfully for over three months after all other means of therapy had failed in a 15-month period, including massive doses of calciferol and AT10 both orally and intramuscularly.

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We are indebted to Drs. Joseph E. Murray, Somers H. Sturgis, John R. Brooks, A. Scott Earle, and Roy Menninger of The Peter Bent Brigham Hospital, and to Dr. Charles E. Dent of The University College Hospital, London, England, for their collaboration and assistance in the many aspects of the care of this patient.

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CORRELATION OF GOITER, DEAFMUTISM AND MENTAL RETARDATION WITH SERUM THYROID HORMONE LEVELS IN NON-CRETINOUS INHABITANTS OF A SEVERE ENDEMIC GOITER AREA IN INDIA*

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ABSTRACT

Serum protein-bound iodine (PBI) levels were determined in 48 subjects in a native village in northeastern India, and in 35 of them butanol-extractable (BEI) levels were also determined. There was a high incidence of endemic goiter, deafness, mutism, deafmutism, mental deficiency and cretinism in the village. The selected group of inhabitants was roughly representative, although only 1 was a cretin. The mean serum PBI and BEI levels in this group were low compared to the mean values for these determinations among normal subjects in the United States. The difference between the mean values for PBI and for BEI was greater in the Indian subjects than in those residing in the United States. Among the Indian subjects: 1) those with goiter had lower mean serum PBI and BEI levels than those without goiter, 2) deaf persons had lower PBI levels than those who could hear, and 3) subjects with mental deficiency had lower PBI and BEI levels than those who were not mentally deficient.

THE World Health Organization Study Group on Endemic Goiter concluded in part in 1953 (1) that "1. Cretinism occurs in areas where goiter has been endemic for long periods. 2. Feeblemindedness, apart from cretinism, rises distinctly in incidence in areas of endemic goiter. Educability is also lowered, with resultant economic loss. 3. Reports have suggested a significant correlation between the incidence of deafmutism and that of

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endemic goiter. Factors other than goiter may cause feeblemindedness or deafmutism, and much more work will be required for critical assessment of such factors in populations."

Since we have found no reports in the literature on the etiology of "Feeblemindedness apart from cretinism . . . " in a severe endemic goiter area, we wish to present our studies in such an area, using serum protein-bound iodine (PBI) and serum butanol-extractable iodine (BEI) levels as the laboratory tests of thyroid function.

METHOD

The general location of the village in which the study was performed is shown in Figure 1. This village was chosen because no artificial source of

FIG. 1. Map showing the location of the native tribes studied in the Northeastern Frontier Agency (N.E.F.A.) of India in the Himalayan Mountains.

iodine is available to its natives, and they exhibited a high incidence of endemic goiter, cretinism, deafness, deafmutism and mental deficiency. The 64 inhabitants selected for investigation had a three-generation family history which provided data for a genetic study to be reported later. Serum PBI determinations were performed in 48 of these 64 subjects, and form the basis of the present report.

In each case a detailed medical history was taken and complete physical examination made. The diagnosis of goiter was made only when the subject had easily visible and palpable thyroid enlargement. The majority of the natives studied did not have goiters and served as "controls." The diagnosis of deafness was easily made by noting the reaction to commands spoken behind the subject; native interpreters assisted in this procedure. The diagnosis of mental deficiency was more difficult, especially in children, but native interpreters assisted in comparing the mentally deficient with normal siblings.

Sufficient blood was obtained in 35 of the 48 subjects for serum BEI determinations. The blood was allowed to clot in flamed tubes. The serum was then pipetted into other sterile tubes. These tubes immediately were sealed under sterile techniques. The tubes were sent by air mail-special delivery to a biochemical laboratory[1] in the United States and arrived there within one to two weeks after the blood was drawn. Serum PBI (2) and BEI (3) determinations were performed immediately.

To check on the possibility that the PBI and BEI content of the sera might decrease because of lack of proper refrigeration of the serum during the shipping period of one to two weeks, blood was drawn and serum obtained on a euthyroid employee in Ann Arbor under identical conditions. The serum was allowed to stand at room temperature during July for three weeks. Aliquots of this serum were pipetted and sent to the same laboratory for analysis immediately, and at intervals of one, two and three weeks after withdrawal of the initial blood specimen. The PBI values on these four lots of serum were 6.9, 6.9, 6.1 and 6.7 μg per 100 ml.

RESULTS

In Table 1 the pertinent data are presented.

Incidence of goiter, deafness, mental deficiency and cretinism

Of the 48 persons, 25 were males and 23 were females, ranging in age from 4 to 60 years (mean age, 29 years). Forty-four per cent had a goiter; 35 per cent were deaf, mute, or deafmute; 23 per cent were mentally deficent; and 2.3 per cent (1 subject) were cretins. Adult males exhibited roughly the same incidence of these defects as the entire group of 48 members, except that the only cretin was an adult male. Adult females had less than half the incidence of deafness, mutism, or deafmutism, and less than half the incidence of mental deficiency shown by the entire group of 48. Among the children, no males had a goiter, and the incidence of deafness, mutism, deafmutism and mental deficiency was strikingly higher than the average for the entire group of 48 members. The female children resembled the male children in these respects, but less strikingly.

Serum PBI and BEI values

In Table 2 are presented the mean serum PBI and BEI values for the group, correlated with the presence or absence of goiter, deafness, mutism and mental deficiency.

1. The mean serum PBI and BEI values for the 48 subjects in India were subnormal as compared to mean normal values for these determinations in the United States of America ($P < 0.1$). The mean serum PBI level for the 48 residents of India was 3.8 μg. per 100 ml. and the mean serum BEI level was 2.5 μg. per 100 ml. compared to the corresponding values for normal subjects in the United States, namely, 5.5 μg. per 100 ml. and 4.9 μg. per ml. (3).

[1] Bio-Science Laboratories, 2231 Carmalina Avenue, Los Angeles 64, California.

TABLE 1. DATA ON 48 SUBJECTS IN A SEVERE ENDEMIC GOITER AREA (INDIA)

Subject No.	Age (yrs.)	Goiter	Deaf, mute or deafmute	Mental deficiency	Cretin	Serum	
						PBI (μg./100 ml.)	BEI (μg./100 ml.)
Adult males							
1	60	X		X		2.4	2.3
2	50	X				2.0	2.1
3	50					3.3	—
4	50					5.1	—
5	45					4.7	2.0
6	45	X				4.4	—
7	45	X	X			3.9	1.8
8	45					4.5	4.0
9	40		X			5.0	—
10	40	X	X			3.6	—
11	38					3.8	1.8
12	38	X				4.3	2.3
13	35					4.0	2.6
14	30		X	X	X	2.4	2.0
15	30	X	X	X		3.2	1.7
16	28	X	X	X	?	2.7	1.0
17	27	X				3.4	1.3
18	27					3.6	3.6
19	25			?		4.0	2.9
20	25	X				3.8	2.2
Adult females							
21	45	X	X	?		1.3	1.3
22	45					4.7	—
23	40					3.2	3.2
24	40	X				3.5	—
25	40					3.2	1.4
26	40	X				4.5	—
27	38	X				4.5	1.5
28	35					4.9	2.1
29	35	X				4.4	2.0
30	30	X	X	X		2.6	2.7
31	30	X	X			2.7	—
32	28	X				4.0	3.5
33	24					3.6	3.1
34	22					7.8	5.7
35	20					6.0	3.6
Male children							
36	10		X	X		5.2	—
37	9					4.4	2.4
38	8		X			3.6	—
39	7		X	X		4.1	3.3
40	4					5.9	—
Female children							
41	18	X	X	X		1.3	1.0
42	15					4.3	4.1
43	12	X				3.4	1.2
44	9		X			2.6	2.6
45	9					5.1	3.4
46	7		X	X		4.1	2.9
47	7		X			3.1	3.1
48	7	X	X	X		1.8	—

TABLE 2. MEAN SERUM PBI AND BEI VALUES CORRELATED WITH THE PRESENCE OR ABSENCE OF GOITER, DEAFNESS AND MENTAL DEFICIENCY

	U.S.A., normal values	Endemic goiter area in India						
		Group as a whole	Goitrous	Non-goitrous	Deaf	Not deaf	Mentally deficient	Not mentally deficient
Mean serum PBI (μg./100 ml.)	5.5	3.88 (48)*	3.22 (21)	4.30 (27)	3.13 (17)	4.22 (31)	2.98 (10)	4.06 (38)
Mean serum BEI (μg./100 ml.)	4.9	2.50 (35)	1.86 (15)	2.99 (20)	2.13 (11)	2.68 (24)	2.11 (8)	2.83 (25)

* Figures in parentheses indicate the number of subjects studied in each category.

2. Subjects with goiter had lower mean PBI and BEI levels than those without goiter ($P < 0.1$).

3. Deaf persons had lower serum PBI levels than those who could hear ($P < 0.1$).

4. Subjects with mental deficiency had lower serum PBI and BEI levels than those who were not mentally deficient. ($P = 0.1$–0.5).

5. The difference between the values for PBI and for BEI was greater in the residents of India than in the residents of the United States.

DISCUSSION

Costa *et al.* (4) found no lowering of serum PBI values among the inhabitants of an endemic goiter area in Italy. These investigators also found no decrease in the serum PBI levels in cretins living in this area, and were at a loss to explain their findings. Querido, Schut and Terpstra (5) observed that the concentration of serum PBI in rats fell to subnormal levels when the rats were fed a severely iodine-deficient diet. We have no direct evidence that iodine deficiency caused the thyroid hormone deficiency observed in the 48 subjects of our study; iodine balance studies have not as yet been performed in this group.

We also have no direct evidence that the mental deficiency observed in our noncretinous subjects was caused by hypothyroidism at a critical period of brain growth. It is well accepted, however, that hypothyroidism early in life can cause mental retardation. It is also generally accepted that a lowered concentration of serum PBI and BEI indicates the presence of hypothyroidism unless some other cause of the lowered values can be

found. Furthermore, it is well accepted that in hypothyroidism there is an increased incidence of deafness, usually of a perceptive type (6). The fact that the subjects who had these defects usually associated with endemic goiter had lower mean concentrations of serum PBI and BEI than did the subjects without such defects suggests that hypothyroidism was at least a contributing cause of these abnormalities, presumably related to poor development of the brain in the absence of sufficient thyroid hormone.

Many other factors, such as consanguinity, also may have been operative in the production of these defects.

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EFFECT OF ORAL LIPIODOL ON THYROIDAL I^{131} UPTAKE AND SERUM PROTEIN-BOUND IODINE CONCENTRATION

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ABSTRACT

Thyroidal I^{131} uptake and serum protein-bound iodine concentration were measured before and at intervals after the administration of Lipiodol by mouth to euthyroid subjects. The thyroidal I^{131} uptake was depressed for 445 days (the duration of the study) after ingestion of Lipiodol. The serum protein-bound iodine level was first markedly elevated, fell rapidly during the first three to four months, and then very slowly approached normal. The serum free iodine level remained high in 2 of 4 subjects for 445 days. These findings indicate that Lipiodol is readily absorbed from the intestinal tract, is stored in an unknown site, and its iodine is slowly released in amounts sufficient for binding with the serum proteins and for suppression of the thyroidal I^{131} uptake.

ORGANIC iodine compounds used in roentgenologic diagnosis have been shown to elevate the level of serum protein-bound iodine (PBI) and/or lower the uptake of I^{131} by the thyroid gland. Under these circumstances, results of the PBI and I^{131} uptake tests are no longer valid indices of thyroid function. It is particularly important to be aware of the iodine compounds which alter these results for long periods of time after their administration; these can be easily overlooked in the patient's history. Lipiodol when used for bronchography or myelography is one such agent, presumably because it is so slowly absorbed from the lungs and the spinal canal. Recent study of a pediatric patient (R.D.) led us to suspect that when Lipiodol is administered orally it may likewise alter the PBI level and thyroidal I^{131} uptake for many months. The present study was undertaken with this in mind.

MATERIALS AND METHODS

Four young males between the ages of 12 and 15 years, 2 with spina bifida (R.S., J.G.) and 2 with progressive muscular dystrophy (J.V., S.V.), as well as a 6-year-old female genetic dwarf (R.D.) were included in this study.

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Thyroidal 24-hour I^{131} uptakes (using a well shielded bismuth gamma-counter) were determined before oral administration of Lipiodol and at intervals thereafter. A dose of 10 μc. of I^{131} was given orally for each determination. The normal 24-hour value in this laboratory ranges from 15 to 50 per cent. Blood samples before, and at intervals after administration of Lipiodol were collected in iodine-free vacutainer tubes. The levels of serum protein-bound iodine (PBI) and total iodine were measured by a modification of the method of Zak *et al.* (1). The difference between the total iodine and the protein-bound iodine is the free iodine (FI) which, according to Astwood *et al.* (2), does not normally exceed 2 μg. per 100 ml. The range of normal PBI levels in this laboratory is 4.0 to 8.7 μg. per 100 ml. The method permits accurate determination of PBI up to 20 μg. per 100 ml.; for concentrations above this level, the sera were appropriately diluted.

Lipiodol, a stable iodine addition product of poppyseed oil containing 540 mg. of iodine per ml., was administered orally. Each subject received 20 ml., except R.D. who received 10 ml.

RESULTS

In the 4 subjects in which it was measured, the thyroidal I^{131} uptake was suppressed immediately after the administration of Lipiodol and remained low throughout the period of observation (Table 1). The elevation in serum PBI concentration was initially very great and the level remained above normal for the duration of the study. The level of serum FI was measured only late in the study, and was found to be elevated in all 4 subjects; at 445 days it was still high in Patients J.V. and S.V., but normal in Patients R.S. and J.G. In Figure 1 the serum PBI concentrations are plotted against time. The curves demonstrate a relatively rapid phase of decrease in the high PBI level during the first three to four months, fol-

TABLE 1. THYROIDAL 24-HOUR I^{131} UPTAKES AND SERUM PROTEIN-BOUND IODINE (PBI) AND FREE IODINE (FI) LEVELS, BEFORE AND AFTER ORAL LIPIODOL

No. of days after Lipiodol	Patient R.S.			Patient J.G.			Patient J.V.			Patient S.V.			Patient R.D.
	I^{131} uptake (%/24 hrs.)	PBI (μg./100 ml.)	FI (μg./100 ml.)	I^{131} uptake (%/24 hrs.)	PBI (μg./100 ml.)	FI (μg./100 ml.)	I^{131} uptake (%/24 hrs.)	PBI (μg./100 ml.)	FI (μg./100 ml.)	I^{131} uptake (%/24 hrs.)	PBI (μg./100 ml.)	FI (μg./100 ml.)	PBI (μg./100 ml.)
0	34	5.7		27	4.6		48	7.5		54	8.3		
5–10	5	685.0		6	386.0		6	1340.0		10	965.0		
15–20		457.5			400.0			765.0			745.0		
25–30	5	270.0		5	245.0		6	560.0		3	570.0		
40–50		180.0			150.0			350.0			305.0		>20.0
60–70	11	115.0		8	59.0		6	232.5		11	129.0		
85–95	13	50.0		13	44.0		13	82.0		13	>20		
119													>20.0
140		26.3			12.3			67.5			30.5		
168													16.6
175–185	5	16.0		7	12.4		7	>20.0		8	>20		
200–205								>20.0			19.2		
250–255		12.2			11.0			44.8			25.8		
275–300	10	11.3	2.5	13	11.7	6.0	10	37.5	4.0	11	30.0	7.5	
340–350	16	11.4	1.2	12	12.0	1.7	10	24.5	10.0	11	28.5	7.0	11.2
440–445	15	10.8	1.9	12	12.1	1.9	12	17.5	8.0	16	21.5	2.5	
504													11.5

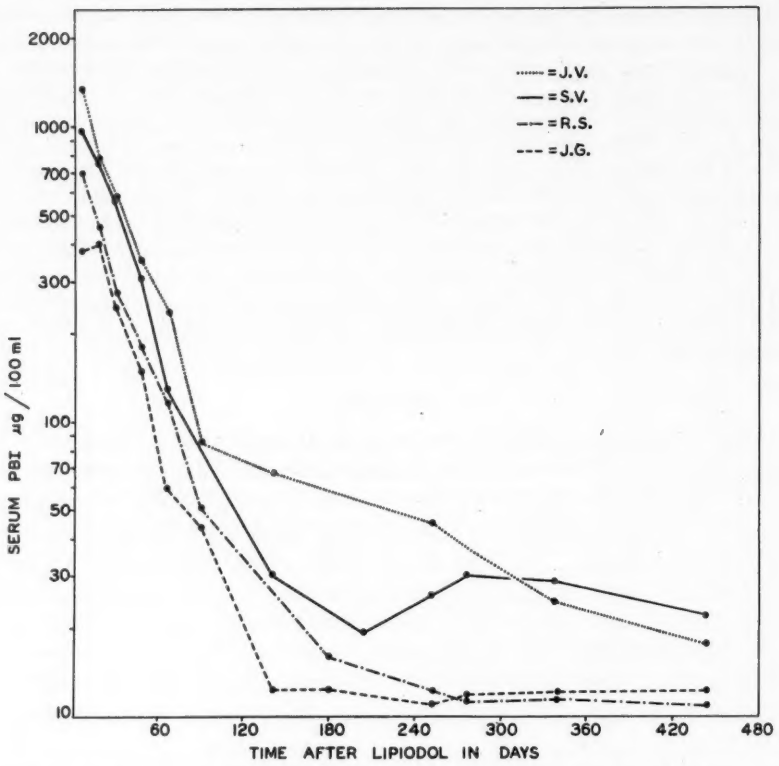

FIG. 1. After administration of Lipiodol the serum PBI levels at first decreased rapidly, and then very slowly approached normal. The concentration of serum PBI was still elevated at 445 days.

lowed by a period when the PBI level decreased very slowly, if at all, over many months. The concentration of PBI decreased less rapidly in the patients with progressive muscular dystrophy (J.V. and S.V.) and remained at higher levels than in the other 2 subjects.

Roentgen studies of the chest and abdomen at the end of the study failed to reveal any radiopaque material.

DISCUSSION

Lipiodol has been used for roentgen visualization of the intestinal tract in young infants, for the detection of suspected tracheoesophageal fistulae, and in the study of the absorption of fat from the gastro-intestinal tract. Groen (3) reviewed the latter subject in 1948 and presented additional in-

formation about the absorption and metabolism of Lipiodol. Silverman and Shirkey (4) simplified a test for studying fat absorption, using Lipiodol; this was further modified by Delory, Israels and Jonasson (5). None of these studies presented data on serum PBI levels or other measures of thyroid function.

Recent studies (6–8) suggest that Priodax and Telepaque do not alter the values for serum PBI and thyroidal I^{131} uptake for prolonged periods of time, particularly in hyperthyroid subjects. Astwood (9) has reported a markedly prolonged half-life of an iodinated compound, Teridax, as indicated by elevated serum PBI levels without suppression of thyroidal I^{131} uptake.

Lipiodol, when used for bronchography or myelography, will cause elevation of the serum PBI concentration for prolonged periods of time. Man and Peters (10) found high values for serum PBI up to nineteen months after bronchography in 3 subjects; in 2, the values for non-precipitable iodine were also high. In 1 subject no dye was seen in the roentgenograms, and the serum non-precipitable iodine level was 6.6 μg. per 100 ml. after eleven months. The authors concluded that deposits of Lipiodol can discharge iodine into the blood stream for an extremely long time, and that part of this iodine tends to precipitate with the serum proteins. The concentration of butanol-extractable iodine in serum is also artifactually elevated by organic iodine (11).

The data presented here indicate that Lipiodol is absorbed, and that its iodine appears in the blood in a form which is readily bound to protein. During the first 100–150 days after administration of Lipiodol by mouth, the serum PBI levels decreased relatively rapidly. A considerable proportion of Lipiodol is probably stored and continues to release iodine slowly in amounts sufficient for binding with the serum proteins as well as for circulating in the free form. The elevated FI level causes a prolonged suppression of thyroidal I^{131} uptake. This is in contrast with the effect of Teridax, which does not suppress thyroidal I^{131} uptake, although the serum PBI level remains elevated for years (9).

These studies suggest that the prolonged effects of Lipiodol on parameters of thyroid function are not limited to the use of the substance in bronchography and myelography, but include oral administration as well. The material is absorbed and stored in an unknown site. There is a continual release of iodine in quantities sufficient to lower the thyroidal I^{131} uptake and elevate the concentration of serum PBI.

Of some interest, but of unknown significance, is the difference between the serum PBI levels of the 2 muscular dystrophy patients as compared to the other 2 males.

Acknowledgment

We are indebted to Dr. Abram Kanoff, Director of Pediatrics, Jewish Chronic Disease Hospital, for permission to study Patients J.V., S.V., R.S. and J.G.

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HORMONAL TREATMENT OF HAND-SCHÜLLER-CHRISTIAN DISEASE: REPORT ON A CASE WITH DISAPPEARANCE OF THE BONE LESIONS

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ABSTRACT

The clinical course of a case of Hand-Schüller-Christian disease treated with cortisone and ACTH is presented. Estrogens and desiccated thyroid were also administered in order to stimulate osteoblastic activity and thus contribute to reconstruction of bone.

Remission of the clinical manifestations and complete disappearance of the bone lesions were observed. About nine months have elapsed since the discontinuance of all therapeutic measures and the remission has been maintained.

ACCORDING to the work of Holm, Teilum and Christensen (1) and Lichtenstein and Jaffe (2), there is a definite relationship between eosinophilic granuloma and the Hand-Schüller-Christian syndrome. These pathologic alterations of the reticulo-endothelial system either assume an isolated osseous or visceral form of benign character (eosinophilic granuloma) or a more serious generalized and malignant form (Hand-Schüller-Christian syndrome).

Flosi *et al.* (3) reported the beneficial effect of the use of adrenocorticotropin (ACTH) in the treatment of eosinophilic granuloma. Thus encouraged, we decided to try hormonal treatment in Hand-Schüller-Christian disease.

Karlén (4), during administration of cortisone to an 18-month-old girl suffering from Hand-Schüller-Christian disease, observed the disappearance of both hepatosplenomegaly and exophthalmos. However, when cortisone therapy had to be interrupted due to development of edema, the general condition of the patient deteriorated and she died.

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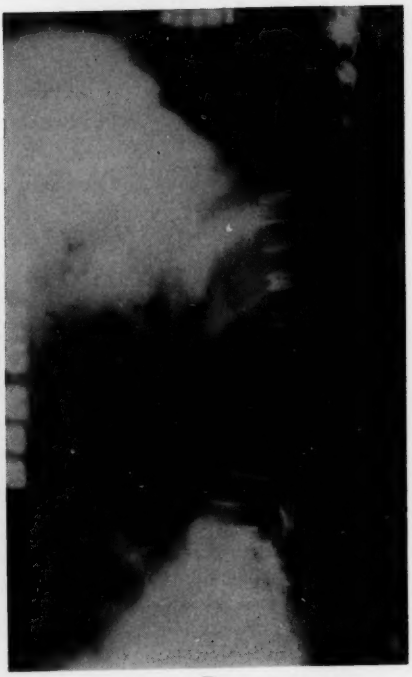
A



B

C

D

FIG. 1

Blahd, Levy and Bassett (5) administered cortisone to an adult male patient with Hand-Schüller-Christian disease and noted general improvement and increased appetite. With the development of edema, the hormonal treatment was replaced with radiotherapy. Domingues, Rodrigues and Sawaya (6) reported a case in a 3-year-old patient treated with cortisone; not only did the diabetes insipidus and hepatosplenomegaly disappear, but there was nearly complete remission of the bone lesions. Avery, McAfee and Guild (7) referred to 2 cases of the disease treated by cortisone therapy with good results.

This paper reports a case of total remission of the bone lesions, which so far has lasted four years.

CASE REPORT

A.C.S. (*H.C. 338734*), a 1-year-old white boy, was admitted on October 16, 1953 to the Department of Otorhinolaryngology. About four months prior to admission his parents had noticed a swelling in his left retroauricular region, associated with suppuration of the left ear, fever, diarrhea and loss of weight. His birth had been normal and his personal and the family background presented nothing of clinical interest.

On physical examination the patient's general condition was poor. He was 69 cm. in height and weighed 6.1 Kg. His temperature was 37.4°C. In both his left retroauricular and right fronto-temporal regions there was a soft swelling. The left external acoustic duct showed granulations in its posterior walls, and fetid suppuration. No exophthalmos was apparent. Slight splenomegaly and hepatomegaly were present, but no adenopathy.

Laboratory data—Negative results with serologic tests for syphilis; concentration of serum total protein 5.5 Gm., albumin 3.7 Gm., and globulin 1.6 Gm. per 100 ml. (Gornall-Bardawill-David); concentration of serum cholesterol 94 mg. (Sheftel), calcium 9.7 mg. (Clark-Collips), and inorganic phosphorus 6.2 mg. per 100 ml. (Fiske and SubbaRow); alkaline phosphatase activity 5.9 King-Armstrong units; negative findings with urinalysis and a Bence-Jones albumin test; no abnormally high urinary volumes.

X-ray examination of the head revealed numerous osteolytic lesions with sharp contours and of various sizes distributed along the superior region of the skull and confluent towards the base (Fig. 1). There were lytic lesions of the left maxilla and on the left iliac and pubic arch. The spine showed lytic lesions of the vertebrae, with collapse of the lower dorsal and lumbar regions (Fig. 1). There were also lytic lesions distributed through the diaphysis of the right femur; the bone appeared expanded and puffed up (Fig. 1).

Biopsy and pathologic examination of the mastoid region revealed a xanthomatous granuloma (Fig. 2). A diagnosis of Hand-Schüller-Christian disease was made.

Clinical course (Table 1). Because of his poor physical condition, the patient was given blood transfusions, glucose, vitamins A and D, sulphadiazine and antibiotics (peni-

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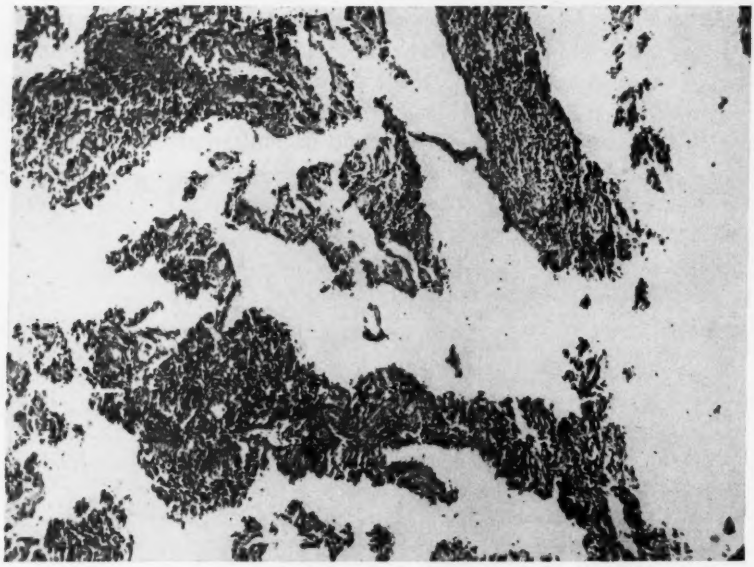
FIG. 1. Roentgenograms taken *before treatment* (Oct. 23, 1953).

A. Numerous osteolytic lesions are distributed along the base of the skull as a whole and destructive lesions are visible on the left arch of the inferior maxillary bone.

B. Collapse of the inferior dorsal and superior lumbar vertebrae.

C. Osteolytic lesions at the level of the left supracondylar region.

D. Medullary destruction along the right femoral diaphysis.



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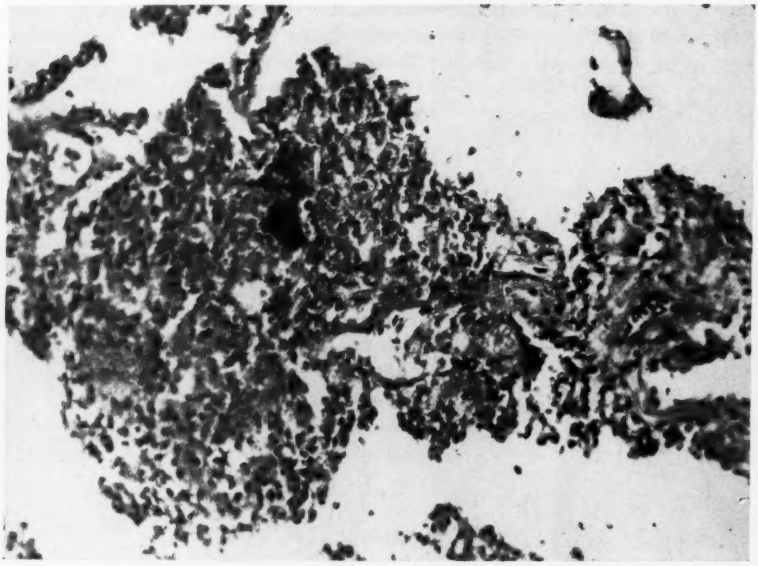

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FIG. 2. **A.** Low-power photomicrograph of the bone biopsy specimen showing inflammatory infiltration with a great number of xanthomatous cells arranged in groups. **B.** High-power photomicrograph of the same specimen shows foamy-appearing cells embedded in a stroma of mononuclear cells. (H. & E. stain.)

TABLE 1. CLINICAL COURSE OF HAND-SCHÜLLER-CHRISTIAN DISEASE IN PATIENT A.C.S.

Date	Wt. (Kg.)	Ht. (cm.)	Bone age	Patient's condition	Bone lesions (x-ray exam.)	Therapy
1953						
Oct. 23	6.1	69		Poor; temp. 37.4° C.	Marked osteolytic lesions (see Fig. 1 and text)	Blood-transfusions, vitamins, antibiotics, sulfadiazine
Dec. 29						Cortisone, 25 mg.q. 6 hrs.
1954	6.4			Poor; temp. 36.5° C.		
Jan. 17				Improved; afebrile	Reduction in lesions (see Fig. 3 and text)	No therapy
Feb. 19	6.6			Poor		Cortisone, 25 mg.q. 6 hrs.
Feb. 27				From Feb. 27 to Apr. 7, improved; appetite returned; active, sitting up in bed and playing		Cortisone, 25 mg.q. 12 hrs.
Mar. 4						Cortisone 25 mg.q. 24 hrs.
Mar. 5						ACTH-gel., 15 I.U. daily
Mar. 25					Further reduction in lesions	Cortisone, 25 mg.q. 24 hrs.
Apr. 3						Cortisone, 15 mg.q. 24 hrs.
Apr. 4						Cortisone, 10 mg.q. 24 hrs.
Apr. 5						Cortisone, 5 mg.q. 24 hrs.
Apr. 7						No therapy
July 6					Almost complete disappearance of the lytic lesions in the skull and lower limbs	
Sept. 1			14 mos.		Relapse of the lesions (see Fig. 4)	Cortisone 12.5 mg.q. 12 hrs. Low-sodium diet supplemented by KCl
Oct. 1						ACTH, 25 mg. i.m. every 8 hrs.
1955						
Jan. 4,						ACTH, 25 mg. i.m. every 12 hrs. Estradiol benzoate, 1 mg. every other day
Feb. 13			20 mos.		Progressive bone reconstruction	
Feb. 24						ACTH, 20 mg. i.m. every 12 hrs. Estradiol benzoate cont'd. Testosterone prop., 25 mg. daily.
Mar. 13						ACTH-gel., 40 I.U. daily. Desicc. thyroid, 50 mg. daily.
Oct.				Definite Cushingoid characteristics (Fig. 5). Gynecomastia (Fig. 6).	Satisfactory resolution	
Nov.				Cushingoid characteristics more pronounced.		ACTH-gel., 40 I.U. every 2 days. Estrone sulphate, 1.25 mg. daily. Desicc. thyroid cont'd.
1956						
June 20						ACTH-gel., 40 I.U., every 3 days. Estrone sulphate & desicc. thyroid con'td
July 25						ACTH-gel., 20 I.U. every 3 days. Estrone sulphate & desicc. thyroid con'td
1957						
Jan.					Almost complete disappearance of the lytic lesions (Mar. 25)	
May 1						No therapy
July 8	20.0	107	8 yrs.	Normal, except for gynecomastia	Complete disappearance of osteolytic lesions; normal osseous structure (see Fig. 7)	

cillin, streptomycin and Aureomycin). His temperature fell and remained at 36.5°C. On October 21, 1953, diarrhea and anorexia developed, and he became pale and weak. Suppuration of the left ear and swelling of the left retroauricular and right fronto-temporal region persisted.

On December 29, 1953, cortisone therapy was started. January 11 marked the beginning of improvement. He began to accept food, and became progressively more ac-

tive. Suppuration of the left ear gradually disappeared and he gained weight (to 6.4 Kg.). Roentgenograms taken January 26, 1954 revealed reduced lytic lesions in the skull with hazy contours and signs of regenerating tissue reconstructing the diploe (Fig. 3). Bone regeneration was also evident on the left side of the jaw. Lytic lesions were still present in the spine, but regenerative processes were also in evidence. There was pronounced regeneration in the lytic areas of the femur as well as signs of newly formed tissue. Cortisone was discontinued on January 17, but resumed on February 19 because of deterioration in the patient's general condition.

From February 27 to April 5, during hormonal treatment (cortisone and ACTH), there was noticeable improvement in the patient's condition. A roentgenogram of the skull taken on March 28 revealed further reduction in the lytic lesions and definite signs of healing. Hormonal therapy was omitted from April 7 to September 1.

A roentgenogram taken September 1 showed a relapse of the lesions (Fig. 4). The deformities of the vertebrae had become more pronounced and collapse was evident, particularly in the dorsal region. Numerous osteolytic foci of irregular and indistinct contours reappeared in the skull. Bone age was estimated to be 14 months.

Again cortisone was administered. On October 1, 1954, ACTH was substituted. On January 4, 1955, estrogens were added, and on March 13 desiccated thyroid was added in order to stimulate osteoblastic activity and bone reconstruction.

X-ray examination in October 1955 revealed a satisfactory resolution of the disease. At this stage the patient necessarily exhibited definite Cushingoid characteristics (Fig. 5). During the following months, the lytic lesions continued to improve gradually and

FIG. 3. Roentgenogram of skull one month after beginning cortisone therapy (Jan. 26, 1954). There is noticeable reduction of the osteolytic lesions.

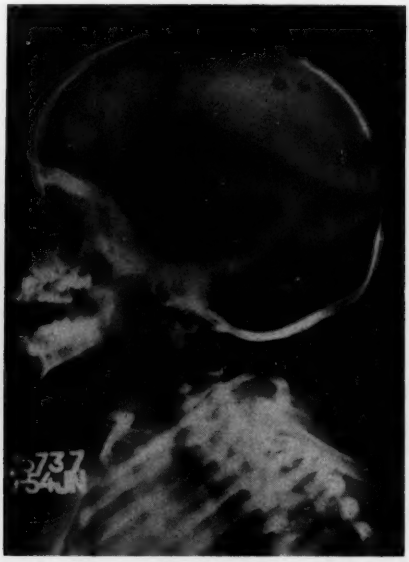


FIG. 4. Roentgenogram of skull after discontinuing therapy (Sept. 1, 1954). Note reappearance of the osteolytic lesions.

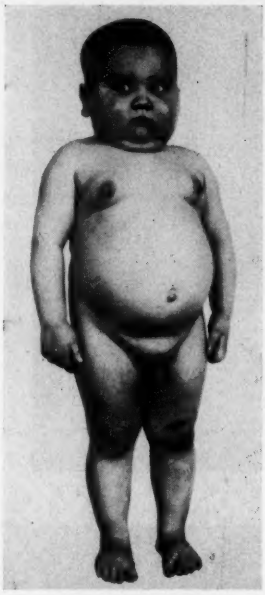


FIG. 5. The patient in October 1955, after almost two years of ACTH and cortisone therapy. At this stage, the patient necessarily displayed Cushingoid characteristics (see text).

FIG. 6. After disappearance of Cushingoid manifestations, a pronounced gynecomastia developed due to estrogen therapy (see text).

the Cushingoid features disappeared when the dose of ACTH was reduced. However, pronounced gynecomastia developed, due to estrogen therapy (Fig. 6).

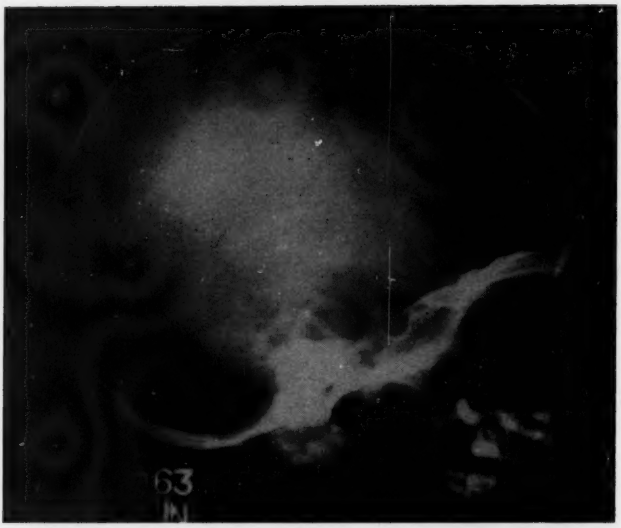
X-ray examination on March 25, 1957, revealed normal bone development and almost complete disappearance of the rarefied zones.

On May 1, 1957, all therapeutic measures were stopped and the patient was kept in the ward under observation. The last X-ray examination, made on July 8, 1957 (Fig. 7), showed normal osseous structure and complete disappearance of bone lesions. Except for the gynecomastia, the patient appeared normal. He was discharged on July 8, 1957, weighing 20 Kg. His height was 107 cm., and blood pressure 90/60 mm. Hg. His bone age (which was about 2 years in September 1954) was now 8 years. Until now (December 1957) the remission has been maintained.

DISCUSSION

The evolution of this case, with complete remission of the clinical manifestations and disappearance of the bone lesions, shows the possibilities for success in the hormonal treatment of Hand-Schüller-Christian disease.

The use of cortisone and ACTH was based on the possible effect of these hormones on the xanthomatous granulomatosis, since they had already



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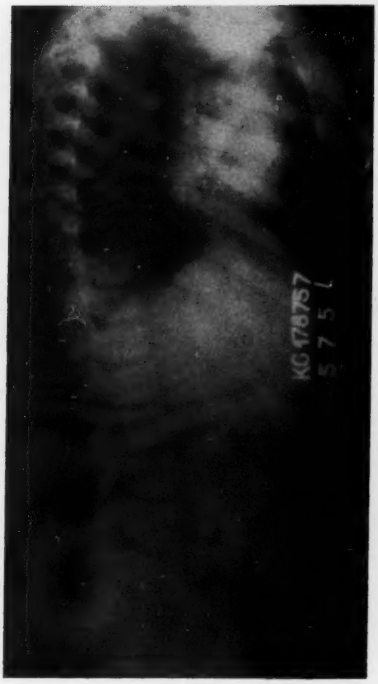

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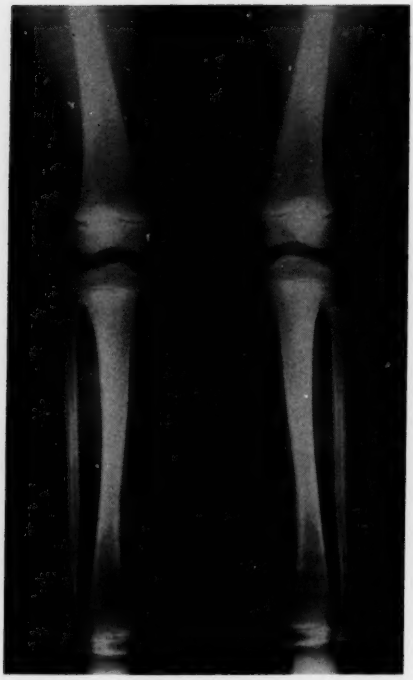

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FIG. 7. Roentgenograms taken *after about three years of hormonal treatment* (July 5, 1957). Note complete disappearance of the osteolytic lesions and the normal osseous structure.

been proved by us to be beneficial in the treatment of eosinophilic granuloma, which is related to the Hand-Schüller-Christian disease.

Estrogens and desiccated thyroid were administered in order to stimulate osteoblastic activity, thus contributing to the reconstruction of bone.

Administration of these hormones was well tolerated, except for the appearance of Cushingoid manifestations during ACTH therapy and of gynecomastia during estrogen therapy. Following a decrease in the dosage of ACTH the Cushingoid features disappeared, but the gynecomastia has persisted up to the present in spite of discontinuance of estrogen. Inhibition of growth was not observed. However a pronounced advance in bone age occurred when thyroid and female sex hormones were added to therapy.

Acknowledgment

We are indebted to Dr. Mario Rubens Montenegro, pathologist of the Department of Pathology of the Faculdade de Medicina of the São Paulo University (Director: Prof. Cunha-Motta).

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FAILURE OF INSULIN TO INDUCE A CHANGE IN RATE OF GROWTH IN A 14-YEAR-OLD BOY WITH ANTERIOR PITUITARY INSUFFICIENCY*

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ABSTRACT

The administration of insulin in a dosage of 60 units daily in combination with desiccated thyroid, ¾ grain, and cortisone, 15 mg., daily for a period of eight months failed to produce a change in the rate of growth of a 14-year-old boy with anterior pituitary insufficiency and dwarfism.

THE failure of growth that accompanies anterior pituitary insufficiency in children is perhaps the most disturbing feature of the disease (1). Efforts to induce satisfactory growth among such children by the use of growth hormones prepared from animal pituitaries or by the use of other hormones have been unsuccessful. Growth hormones prepared from human pituitary glands (2) have recently been demonstrated to produce growth in pituitary dwarfs but such preparations were not available at the time of our study.

In 1953 Salter and Best (3) demonstrated significant growth in hypophysectomized rats following administration of insulin. In their experiments, increasing doses of slow-acting (protamine zinc) insulin were administered to previously hypophysectomized rats while similar groups of animals served as controls or were treated with growth hormone. The insulin-treated animals grew significantly but less than did animals treated with growth hormone, whereas the untreated animals did not grow at all. These experiments prompted us to study the effects of insulin administration in a boy considered to have anterior pituitary insufficiency with dwarfism.

REPORT OF CASE

A boy born on May 2, 1941, the product of a normal pregnancy and spontaneous breech delivery, was first seen at the Mayo Clinic on December 18, 1950, at the age of 9 1/12 years, because of short stature. His birth weight was 6 pounds, 6 ounces (2.9 Kg.). As an infant he ate well, he was alert and his motor development was normal. However, he failed to grow normally, and weighed only 12 pounds (5.4 Kg.) at the age of 1 year.

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* The authors wish to acknowledge the assistance of Dr. R. M. Salassa in the metabolic study of this patient.

The usual childhood diseases were uncomplicated. The family history was noncontributory.

Physical examination disclosed an alert, co-operative and cheerful boy whose height was 39½ inches (100 cm.) and weight 36 pounds (16.3 Kg.). The blood pressure was 90 mm. Hg systolic and 70 mm. diastolic. Aside from his immature appearance and small size, the results of general examination were not remarkable. Dental development was delayed and there was congenital absence of several teeth. A diagnosis of dwarfism secondary to anterior pituitary insufficiency was made, and the administration of desiccated thyroid (U.S.P. strong), ¼ grain (0.016 Gm.) daily, was started.

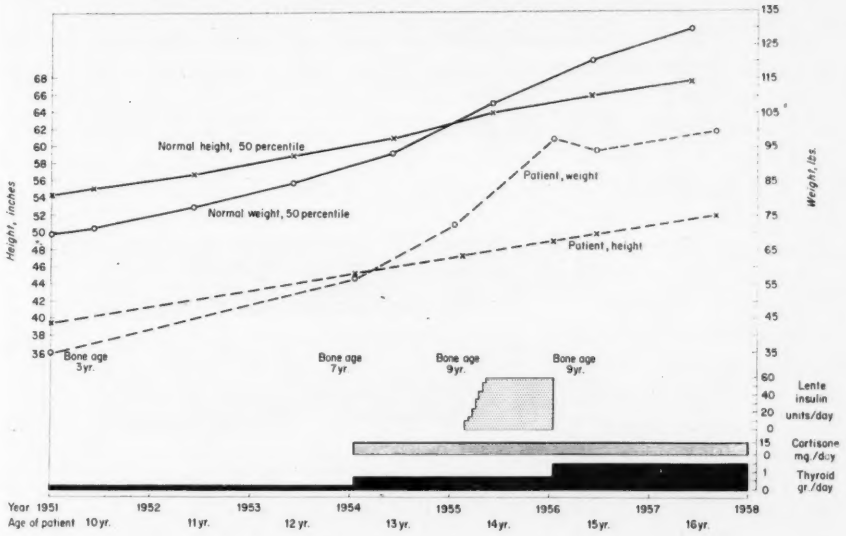

FIG. 1. Data on height and weight of patient during period of observation. Note that the patient gained weight during the period of insulin administration but that there was no change in the rate of growth.

In December 1953, the dosage of desiccated thyroid was increased to ¾ grain a day, and treatment with cortisone (5 mg. every eight hours) was started. In January 1955, at the age of 13 9/12 years, the patient was returned to the clinic because his rate of growth had not changed and he manifested no evidence of sexual maturation. He had had an appendectomy in March 1954, without complication. Figure 1 shows his height and weight during the years 1951 through 1957.

Administration of adrenocorticotropic hormone (Acthar gel) in a dosage of 40 units every twelve hours for five days was followed by a prompt rise in the urinary excretion of 17-ketosteroids from pretreatment levels of 0.8–1.0 mg. per twenty-four hours to levels of 7.9–10.1 mg. by the third, fourth and fifth days. The urinary excretion of 11-oxycorticosteroids was also increased from pretreatment levels of 0.29–0.49 mg. per twenty-four hours to levels of 1.37–2.91 mg. on the third, fourth and fifth days. The level of plasma protein-bound iodine rose from 2.8 μg. per 100 ml. to 6.1 μg. twenty-four hours after a single intramuscular injection of 10 units of purified thyrotropic hormone. A glucose-tolerance test with 50 Gm. of glucose by mouth yielded the following results: fasting

blood sugar concentration, 82 mg. per 100 ml.; blood sugar concentration after one hour, 130 mg.; after two hours, 117 mg.; and after three hours, 84 mg. Administration of regular insulin in doses of 5, 10 and 15 units at 8 A.M. on successive days did not produce hypoglycemic attacks. The concentration of blood sugar determined at intervals up to eight hours after the injections of insulin was more than 72 mg. per 100 ml. in each instance.

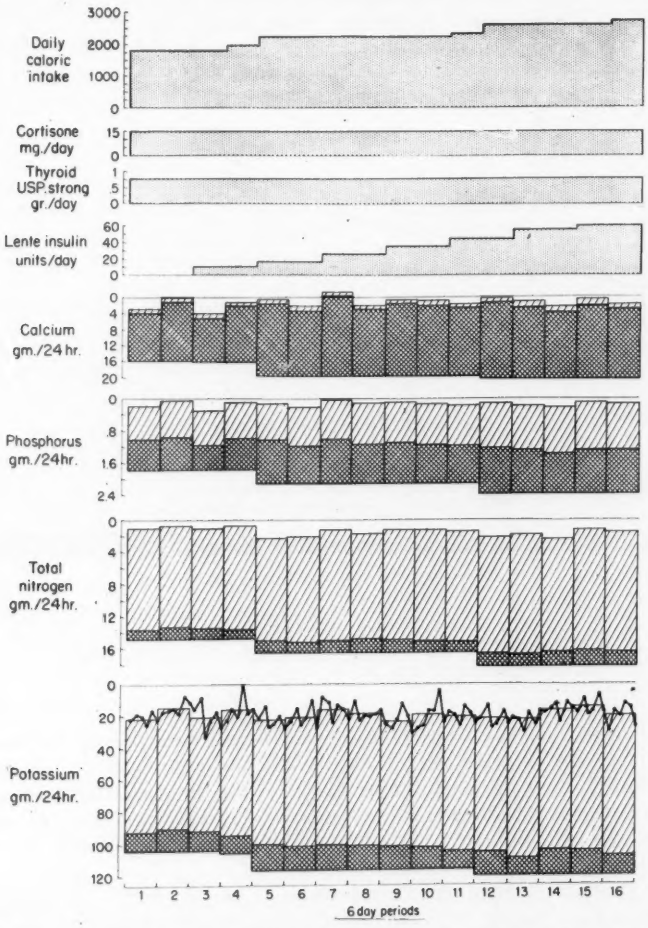

FIG. 2. Effect of insulin administration on caloric intake and on calcium, phosphorus, nitrogen and potassium balances. Balance data are charted in the accepted fashion: the daily intake is charted from the 0 line down, and the average daily excretion is charted upward from the bottom line. Fecal excretion is indicated by dark shading and urinary excretion by light shading. A negative balance is indicated by the column above the 0 line and a positive balance by the unshaded space immediately below the 0 line. Daily variations of potassium balance are indicated by the points connected by the heavy line.

METHODS AND RESULTS OF SPECIAL STUDY OF PATIENT

This patient was studied in a series of metabolic balance periods, each period being six days long. He was allowed to select his own diet from 3 menus which were used in rotation. During the course of the study it was necessary to increase the caloric content of the diet, as indicated in Figure 2. A preliminary period of observation was followed by 2 control periods of six days each, after which the patient received an increasing daily dose of lente insulin (U 40). A daily dose of 60 units was given during the last 2 six-day periods and for a period of eight months after the study was completed. Through the entire study the patient was given thyroid, $\frac{3}{4}$ grain (0.05 Gm.), and cortisone, 15 mg., daily. Although cortisone in small doses has been demonstrated to depress the rate of growth of patients with pituitary dwarfism (4), its administration was considered necessary to prevent hypoglycemia. It should be noted that the subject of this study received cortisone in the same dosage for fifteen months before, and for almost two years after insulin administration, during which time the rate of growth was not altered. The study and analyses were carried out in the same manner as were previously described studies at this institution (5).

The results pertinent to this discussion are shown in Figure 2. It can be seen that the nitrogen, calcium, phosphorus and potassium balances during the periods insulin was administered were not significantly different from those observed during the control periods. Values for serum electrolytes including sodium, potassium, chloride, calcium and inorganic phosphorus were normal throughout the study, as were the hematocrit readings and the values for alkaline phosphatase and blood urea. The patient had an increased desire for food and the daily caloric intake rose. He gained weight during the period insulin was administered and lost weight when administration was discontinued. Mild hypoglycemia occurred on a few occasions during the insulin period and was always promptly relieved by the feeding of carbohydrates. Administration of insulin over a period of eight months did not change the rate of growth, as shown in Figure 1.

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ELECTROPHORETIC ALTERATIONS IN SERUM PROTEINS OF PATIENTS WITH HIRSUTISM

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ABSTRACT

In 17 young women suffering from hirsutism, the pattern of serum proteins was studied by paper electrophoresis. Weekly determinations were also made of urinary 17-ketosteroids and 17-hydroxycorticosteroids. There was no correlation between increments in the level of serum gamma globulin and changes in the urinary excretion of 17-hydroxycorticosteroids. In 9 of the 10 patients in whom the level of serum gamma globulin level was elevated there was also an intermittent elevation in the excretion of urinary 17-ketosteroids. This seems to indicate that hypergammaglobulinemia in hirsutism is caused by an excess of endogenous androgen.

SEVERAL authors have studied the action of corticotropin (ACTH) and various steroids on the pattern of serum proteins. Dougherty and White (1) noticed an increase of gamma globulin in animals treated with adrenal extracts and ACTH, which they ascribed to the lysis of leukocytes produced by these compounds. Sachs and co-workers (2) found an increase in the level of α_2-globulin in women treated with ethinyl-19-nortestosterone and androstenediol. Bernasconi (3) reported increments in the level of serum gamma globulin in rats treated with ACTH and prolactin. These anomalies led us to study the serum proteins in hirsute women.

MATERIAL AND METHODS

Seventeen young women from 18 to 32 years of age were studied. They exhibited moderate or pronounced hirsutism of the face, limbs, breasts, and abdomen, associated with varying degrees of acne.

Urinary 17-ketosteroids were measured in all subjects, weekly during a control period, and then following an eight-hour intravenous injection of 25 I.U. of ACTH. Assays were carried out according to Ricca's method (4); with this technic normal values for women vary between 4 and 10 mg. per twenty-four hours. Urinary 17-hydroxycorticosteroids (Porter and Silber) were measured in the resting condition and after stimulation with 25 I.U. of ACTH, by the method of Ricca, Argüelles and Chekherdemian (5).

Electrophoretograms were obtained with Evans Electroselenium Harlow equipment, Hobunt power source, 230 volts a.c., 50 cycles. Schleicher and Schüll paper was

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used, in strips of 35 cm. × 5 cm. The buffer was veronal, veronal sodium; ionic power 0.1, pH 8.6, running twenty-four hours. The strips were colored with bromophenol blue and read after elution with 0.04 per cent aqueous sodium hydroxide. A confirmatory reading by sensitometry (Eel scanner) was carried out after the strips were exposed to ammonia vapors and cleared with glycerin. The correcting factor corresponded to coloration with bromophenol blue: 1.6.

RESULTS

Gynecologic study

Clinical examination and pelvipneumography showed that in 10 out of the 17 hirsute women studied, the ovaries were polycystic.

Serum proteins

In 10 of the 17 patients there was an appreciable increment in the proportion of gamma globulin in the serum, ranging between 22 and 29 per cent (normal maximal limit, 20 per cent). (Table 1).

In 9 cases there was also an increase in the proportion of α_2-globulin, the values ranging between 11 and 13 per cent (normal maximal limit, 8 per cent). An increase in both α_2-globulin and gamma globulin occurred in only 5 cases (Table 1).

Urinary 17-ketosteroids

In all the women with high serum gamma-globulin levels, the values for urinary 17-ketosteroids (17-KS) were above normal—usually between 16

TABLE 1. SERUM PROTEINS; ELECTROPHORETIC VALUES

Case No.	Protein (Gm./100 ml.)	Albumin		Globulin									
				Total		α_1		α_2		β		γ	
		%*	Gm./100 ml.	%	Gm./100 ml.	%	Gm./100 ml.	%	Gm./100 ml.	%	Gm./100 ml.	%	Gm./100 ml.
1	6.70	36	2.410	64	4.290	6	0.400	13	0.870	16	1.070	29	1.940
2	5.92	45	2.660	55	3.260	4	0.236	8	0.472	16	0.944	27	1.595
3	8.10	53	4.300	47	3.800	3	0.243	7	0.567	11	0.891	26	2.107
4	6.62	39	2.580	61	4.040	6	0.400	11.5	0.760	18	1.190	25.5	1.670
5	6.50	43	2.800	57	3.700	10.6	0.690	9	0.585	12.4	0.805	25	1.625
6	6.76	38	2.570	62	4.190	9	0.607	11	0.745	17	1.150	25	1.690
7	6.45	51	3.290	49	3.150	5	0.320	9	0.580	12	0.770	23	1.480
8	6.76	50.5	3.420	49.5	3.340	3	0.201	6	0.402	17	1.150	23.5	1.590
9	6.76	45	3.040	55	3.718	7	0.474	11	0.742	14	0.945	23	1.557
10	8.70	48	4.183	52	4.517	1	0.087	13	1.130	16	1.390	22	1.915
11	6.76	41.5	2.800	58.5	3.960	9.5	0.642	13	0.880	16	1.080	20	1.350
12	7.76	48	3.720	52	4.040	9	0.697	12	0.854	11	0.932	20	1.550
13	7.80	53	4.130	47	3.670	7	0.560	11	0.850	11	0.850	18	1.400
14	8.00	50.5	4.040	49.5	3.960	8.5	0.680	11.5	0.920	11.5	0.920	18	1.440
15	6.76	56	3.790	44	2.976	6	0.406	8	0.540	13	0.880	17	1.150
16	6.40	51.5	3.300	48.5	3.100	6.4	0.410	9.4	0.602	17.4	1.114	15.3	0.980
17	6.76	56	3.781	44	2.979	8	0.541	10	0.677	12.5	0.846	13.5	0.915

* Per cent of total protein.

TABLE 2. COMPARISON BETWEEN INTENSITY OF HIRSUTISM, SERUM α_2-GLOBULIN AND GAMMA-GLOBULIN LEVELS, AND URINARY 17-KS AND 17-OHCS

Case No.	Intensity of hirsutism	Serum γ-globulin*	Serum α_2-globulin*	Higher-range urinary 17-KS (mg. 24 hrs.)	Lower-range urinary 17-KS (mg. 24 hrs.)	Polycystic ovaries	Urinary 17-OHCS (mg. 24 hrs.)	Urinary 17-OHCS after ACTH (mg. 24 hrs.)
1	++	29	13	33	20	−	4	15.3
2	++	27	8	18.5	13.2	−	5	9.7
3	+++	28.5	7	26	21	−	3.2	18.8
4	+	25.5	11.5	16	11	−	4	36.0
5	++	25	9	15	8	−	9	26.0
6	+++	25	11	14	4	+	4	14.0
7	++	23	9	30	21	−	4	11.5
8	+++	23	6	15	8	+	6	20.1
9	++	23	11	19	7	+	2	4.1
10	++	22	13	16	13	−	—	—
11	++	20	13	15	4	+	5	23.0
12	++	20	12	10	2	+	5	10.0
13	+++	18	11	9	4	+	6	14.0
14	+++	18	11.5	9	2	+	2.8	4.3
15	+	17	8	13.5	8	+	6	29.0
16	++	15	9	4.8	0.6	+	—	—
17	++	13.5	10	9.6	2.1	+	—	—

* Percentage of total protein.

and 33 mg. per twenty-four hours, but sometimes there were wide fluctuations. On the other hand, among the 7 women whose gamma-globulin levels were normal, in only 2 were the urinary 17-KS increased.

Urinary 17-hydroxycorticoids

Basal urinary 17-hydroxycorticosteroid (17-OHCS) excretion was normal in all patients. Responses to stimulation with intravenous ACTH were very high in Cases 2 and 4 (see Table 2). In these 2 cases there was also a significant increment in the serum gamma-globulin level.

In Cases 3 and 14, with a very high level and a normal level of serum gamma globulin respectively, there was a poor response to ACTH.

In the remaining cases with high levels of serum gamma globulin, urinary 17-OHCS excretion was normally increased by administration of ACTH. The results are listed in Table 2.

DISCUSSION

Of 17 hirsute women whose serum protein patterns were studied, 10 showed anomalies consisting of an inversion of the albumin/globulin ratio

and an increase in the proportion of gamma globulin, which fluctuated from 10 to 90 per cent of the maximal normal limit.

The serum α_2-globulin level, which Sachs and co-workers (2) found to be elevated after administration of 18-nortestosterone and methylandrostenediol, was increased in only 9 of our 17 patients. No parallelism could be observed between the magnitude of the changes in serum α_2-globulin and urinary 17-KS.

The 7 hirsute women with normal levels of serum gamma globulin had polycystic ovaries (radiologically diagnosed); moreover, their urinary excretion of 17-KS was usually normal and fluctuated only slightly.

Among the 10 patients with high levels of serum gamma globulin, urinary 17-KS were usually high but varied widely in different determinations. Similar findings have already been reported by us (6), and would seem to be characteristic of hirsutism of suprarenal origin. In 3 patients of this subgroup who had moderately elevated serum gamma globulin levels and high urinary 17-KS excretion, the polycystic ovaries were evident radiologically. In these cases, there were mixed adreno-ovarian anomalies.

Because of the possibility that the abnormal electrophoretic patterns could be due to a hepatic disturbance, flocculation tests (Hanger, thymol), were carried out in all cases. Results were positive in only 1 case (No. 3); this patient had a high excretion of urinary estrogens (218 gamma per day of total estrogens, of which 180 gamma pertained to estrone). Thus hepatic insufficiency was an improbable explanation for the changes in the serum proteins.

Dougherty (1) and Bernasconi (3) suggested that the increment in serum gamma globulin levels in animals is produced by the action of glucocorticoids (injected or segregated because of stimulation by exogenous ACTH) in destroying leukocytes, with consequent liberation of globulin. In our series, 2 of the 4 women with the highest levels of serum gamma globulin reacted to ACTH with corticoid excretions that were higher than normal. Nevertheless, since this happened in only 2 cases out of 10, this observation does not establish confirmation of the preceding theory. On the other hand, the correlation between the increments in the level of serum gamma globulin and in urinary excretion of 17-KS in this group of hirsute women is outstanding, and would indicate that in hirsutism hypergamma-globulinemia is the result of dysproteinemia induced by an excess of circulating androgens. This relationship constitutes a sign of importance in the diagnosis of suprarenal hypertrichosis ("idiopathic hirsutism"). According to our knowledge, it has not been described to date.

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ACID MUCOPOLYSACCHARIDE IN THE THYROID GLAND*

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ABSTRACT

Acid mucopolysaccharide was demonstrated in thyroid tissue and its concentration measured by carbazole and orcinol determinations of the hexuronic-acid component. One lobe of the thyroid gland of each of 8 dogs was removed surgically, studied histologically, and analyzed chemically. In 6 of these dogs, the second lobe was removed after thyroid-stimulating hormone (TSH) had been administered subcutaneously for two to three days; the 2 dogs not treated with TSH served as controls. Chemical assay demonstrated an increase in mean values for hexuronic-acid in mucopolysaccharide of 61 per cent after 20 units of TSH given over a two-day period, 115 per cent after 30 units of TSH given over a three-day period, and 70 per cent after 40 units given over a two-day period, using the carbazole determination. Mean increases of 58, 95 and 82 per cent, respectively, were found using the orcinol determination. Histologically, these glands showed approximately a 40 per cent increase in follicular cells, a 40 per cent decrease in colloid, but no noticeable change in the area occupied by stroma. Mast cells increased in number and size and were concentrated around vessels in the lobes removed after treatment with TSH.

Twenty-seven specimens of human thyroid gland were obtained at the time of surgical removal. No consistent relationship was demonstrated between mucopolysaccharide content and the nature of the thyroid disease.

THYROID tissue has been reported to be rich in glycoprotein and poor in mucopolysaccharide (1). By means of a recently described practical analytical method, acid mucopolysaccharide has been demonstrated in thyroid tissue, and its concentration measured (2). Using this method of assay, thyroid-stimulating hormone (TSH) has been found to produce an increase in the concentration of acid mucopolysaccharide in the thyroid gland of the dog. Variations in acid mucopolysaccharide concentration in diseases of the human thyroid gland have also been studied.

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METHOD

Dog thyroid gland

One lobe of the thyroid gland was removed surgically from each of 9 mongrel dogs under intravenous sodium pentothal anesthesia. The second lobe was removed ten to fourteen days later. A small piece of each lobe was fixed for histologic section and the remaining tissue was kept frozen until analyzed chemically.

One week after the first lobectomy, TSH[1] was injected subcutaneously in varying daily dosage (Table 1) into 6 of the dogs, starting a few days before the second lobectomy; the last dose was given one day before the second operation. The 2 control dogs received no TSH during the interval between lobectomies. The ninth dog received TSH but the thyroid lobes were used only for histologic study.

TABLE 1. EFFECT OF TSH ON ACID MUCOPOLYSACCHARIDE CONTENT OF SURGICALLY REMOVED LOBES OF THYROID GLAND IN THE DOG

Results are expressed as micrograms of uronic acid in mucopolysaccharide fraction, per 100 mg. of tissue, using carbazole and orcinol methods of determination.

Number of dogs	Lobe 1 Acid mucopolysaccharide			Units of TSH admin'd between lobectomies	Lobe 2 Acid mucopolysaccharide		
	Carbazole method (μg./100 mg.)	Orcinol method (μg./100 mg.)	Ratio, carb./orc. values		Carbazole method (μg./100 mg.)	Orcinol method (μg./100 mg.)	Ratio, carb./orc. values
2	81	106	0.8	0	81	98	0.8
1	59	76	0.8	10, 10	95	120	0.8
2	53	98	0.5	10, 10, 10	114	191	0.6
3	47	97	0.5	20, 20	80	176	0.5

Human thyroid gland

Twenty-seven specimens of human thyroid gland were obtained at the time of surgical removal. Part of each gland was kept frozen until analyzed. Each of the patients from whom these glands were removed belonged in one of the 6 categories of thyroid conditions listed in Table 2, on the basis of clinical features and histologic structure.

Chemical analysis of thyroid tissue

The method by which the tissue was analyzed for acid mucopolysaccharide has been reported (2). Values are expressed as micrograms of uronic acid in acid mucopolysaccharide per 100 mg. of dried fat-free tissue, using carbazole and orcinol determinations. Variations in replicate determinations on individual specimens averaged ±7 per cent (2).

Histology

The authors independently reviewed coded microscopic sections of the dog thyroid glands. The percentage of the cross section occupied by follicular colloid, by follicular

[1] Thytropar, Armour.

cells, and by stroma was estimated without knowledge of which dog or thyroid lobe was represented by each slide.

Half of each thyroid lobe of one of the dogs given 40 units of TSH was fixed part in Carnoy's solution and part in Bouin's solution for special staining techniques.[2] The sections were stained with Masson's trichrome stain, azure A, and periodic-acid Schiff. Mast cells were counted in each of the lobes removed.

TABLE 2. ACID MUCOPOLYSACCHARIDE CONTENT OF HUMAN THYROID GLAND

Results are expressed as micrograms of uronic acid in mucopolysaccharide fraction, per 100 mg. of tissue, using carbazole and orcinol methods of determination.

Diagnosis	Number of patients	Uronic acid (μg./100 mg.)				Ratio of carbazole to orcinol values	
		Carbazole method		Orcinol method			
		Mean	Range	Mean	Range	Mean	Range
Normal thyroid	6	135	106–156	121	98–137	1.09	0.90–1.77
Exophthalmic goiter	4	147	106–167	194	154–224	.78	0.47–1.10
Colloid ad. goiter	8	187	125–280	207	114–282	1.02	0.88–1.32
Carcinoma	5	189	127–285	179	82–257	1.07	0.49–1.60
Hashimoto's Struma	3	76	37–114	115	100–130	.7	.3–1.1
Chronic thyroiditis	2	228	89–367	285	201–369	.8	.4–1.0

RESULTS

Dog thyroid gland

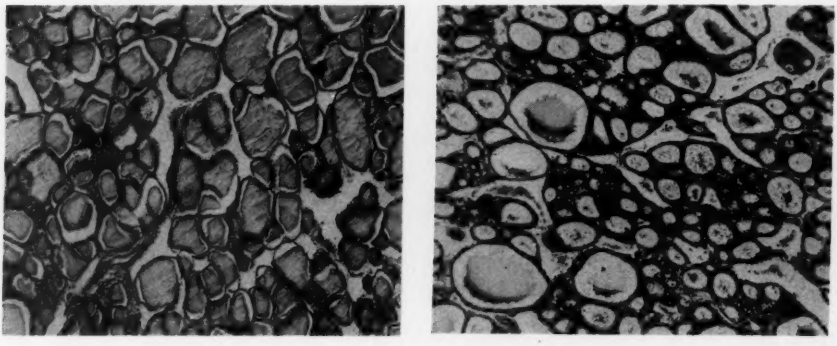
In Table 1 are listed the values for acid mucopolysaccharide concentration in the 16 lobes of 8 dog thyroid glands removed in 2-stage thyroidectomies. In 6 instances the dogs received TSH between the lobectomies.

In the 2 control dogs not receiving TSH, the mean value for the uronic acid content of the acid mucopolysaccharide fraction of the first thyroid lobe removed was 81 μg. per 100 mg. of tissue using the carbazole method, and 106 μg. per 100 mg. using the orcinol method; the ratio of carbazole values to orcinol values was 0.8. Similar levels were found in the second

[2] Courtesy of William C. Castor, M.D., University Hospital, Ann Arbor, Michigan.

lobe of the thyroid in these 2 dogs, indicating no significant changes as a result of the first operation with respect to endogenous TSH stimulation.

When TSH was administered between operations, a significant rise in the total acid mucopolysaccharide concentration was found in the second lobe. The increases in mean values for the carbazole determinations were 61 per cent when a 20-unit dose of TSH was given over a two-day period, 115 per cent when a 30-unit dose was given over a three-day period, and 70 per cent when a 40-unit dose was given over a two-day period. The corresponding increases in the mean values for the orcinol determination were 58, 95 and 82 per cent. There were no changes in the ratio of carbazole values to orcinol values.



A B

FIG. 1. Dog thyroid gland, showing increase in follicular cells and decrease in colloid following administration of TSH. Areas of stroma remained constant. **A.** Before TSH. **B.** After TSH.

Estimates of the percentage of each coded histologic section occupied by colloid, by cells, and by stroma agreed within 10 per cent. Histologically, administration of TSH resulted in approximately a 40 per cent increase in follicular cells and a 40 per cent decrease in colloid (Fig. 1, A and B). The area occupied by stroma remained fairly constant.

Mast cell counts in sections stained with azure A showed an average increase from 3.5 mast cells per low-power field in the control lobe to 7.7 per low-power field in the lobe removed after TSH treatment. The mast cells also increased in size after TSH. They were present in highest concentration around vessels. No significant amount of interstitial mucopolysaccharide was detected in the histologic sections. Masson's trichrome stain showed an increase in size and number of the follicular cells. Both cytoplasm and nuclei showed this increase in size. The cytoplasm became largely fuchsinophilic following administration of TSH.

Human thyroid gland

Table 2 presents results of analyses of human thyroid tissue for acid mucopolysaccharide. No consistent relationship was demonstrated between mucopolysaccharide content and disease state.

DISCUSSION

Previous studies have suggested that there is a hyaluronidase-labile material in colloid which may influence diffusion of hormone into the blood. De Robertis (3) showed that the viscous colloid of normal guinea-pig and rat thyroid became more fluid when the thyroid gland was activated by TSH, and less fluid when the activity of the organ was depressed. These changes were related to the activity of a proteolytic enzyme, cathepsin, found in intrafollicular colloid. Levine (4) observed that extracts of the thyroid gland of the pig, rat and guinea pig contained almost as much spreading-factor activity as extracts of testis. He also noted that an extract rich in hyaluronidase activity, when injected into a thyroid follicle, caused a sudden fall in viscosity of the colloid. Using histochemical techniques, Gersh (5) found that thyroid colloid in rats contained at least 2 protein complexes involving groups which yielded positive results with tests for carbohydrate. One complex appeared to be identical with thyroglobulin, and a smaller fraction was digested by testicular hyaluronidase. In addition he observed cytoplasmic granules containing glycoprotein, which he thought might constitute an essential feature of a Golgi apparatus of follicular cells. He suggested that submicroscopic particles containing glycoprotein may be present in the cytoplasm.

Although Boas and Foley (1) found no uronic acid in mucopolysaccharide by electrophoretic analysis of extracts of thyroid, Bollet (2) demonstrated acid mucopolysaccharide in human thyroid tissue by chemical isolation. He observed that the average concentration of acid mucopolysaccharide in thyroid was higher than in subcutaneous tissue or dermis, but that only 1 per cent of the total hexosamine content of thyroid tissue was in acid mucopolysaccharide. Most of the mucopolysaccharide could be digested by testicular hyaluronidase. Chromatography in 2 systems after chemical separation revealed the presence of 3 components with mobilities resembling chondroitin sulfate, hyaluronate and heparin, but the nature of the individual polysaccharide was not definitely established (2).

In the present series, a considerable increase in the concentration of acid mucopolysaccharide in the thyroid occurred during treatment with TSH. Histologically, the change in mucopolysaccharide content correlated best with the increase in cell mass. In dogs receiving TSH in the interval between lobectomies, the second lobe was approximately twice the volume and weight of the first lobe, regardless of which lobe of the gland was re-

moved first. The second lobe also exhibited a marked increase in vascularity. It is possible that the increase in mucopolysaccharide might be related to this rich vascular network.

The data do not enlighten us regarding the mechanism whereby the production of acid mucopolysaccharide is influenced by TSH. Histochemical studies demonstrated an increase in the number and size of mast cells. This does not prove, however, that TSH has a specific effect on mast cells or that the mast cells control the elaboration of the mucopolysaccharide found by chemical analyses, since other histologic changes were noted after TSH stimulation, namely, increased vascularity of the thyroid, and an increase in the size and number of follicle-cell nuclei.

In human thyroid disease, no specific pattern of concentration of acid mucopolysaccharide was observed. In this study, the histologic structure of dog thyroid gland was more uniform than that of human thyroid gland; thus the fairly large range of values for human thyroids may be related to greater variation in acid mucopolysaccharide concentration from one area to another. A check of 2 adjacent areas of the same thyroid gland in 3 patients yielded differences of 11, 16 and 16 per cent, respectively. It is possible that a greater variation in range could be found in any one gland if samples were taken from more widely separated areas. The fact that the assumed level of TSH stimulation in patients with various thyroid diseases could not be correlated with the acid mucopolysaccharide content of the thyroid gland might also be due, at least in part, to variations in the preoperative treatment and duration of the disease. Although the acid mucopolysaccharide content of dog thyroid gland seems to be a relatively sensitive indicator of the effect of TSH in that animal, we must conclude that in the human subjects studied there was no demonstrable correlation between thyroidal acid mucopolysaccharide concentration and the nature of the disease.

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Letters to the Editor

FAILURE TO DEMONSTRATE HYDROCORTISONE AND ALDOSTERONE DURING PREGNANCY IN ADDISON'S DISEASE

To the Editor:

There are several reports in the literature of increased levels of urinary 17-ketosteroids and corticosteroids in the urine of pregnant women with Addison's disease (1–4). These changes are not invariably found in pregnancy complicated by Addison's disease (5), but when present, they have prompted the assumption (4) that the compounds measured might be degradation products of steroids secreted by either the placenta or by adrenocortical remnants. These steroids, it has been supposed, might account for the so-called protective effect of pregnancy against adrenal crises in Addisonians (6). During the past two years, studies in which more specific methods of steroid analysis were used have failed to demonstrate the metabolic products of hydrocortisone and aldosterone in pregnant women with Addison's disease (7, 8). This report is offered as further evidence against the secretion of hydrocortisone and also of aldosterone in pregnancy associated with primary adrenocortical insufficiency.

M.K. and R.L., multiparous females 29 and 31 years old respectively, were each followed closely through 2 pregnancies. Primary adrenocortical insufficiency was suggested clinically in both patients by asthenia, weight loss, the presence of healed pulmonary tuberculosis, hypotension, increased pigmentation of the skin, and hyponatremia. The diagnosis was confirmed by subnormal levels of urinary 17-ketosteroids (9) and 17-OH-corticosteroids (Porter-Silber chromogen after beta-glucuronidase hydrolysis (10)) and by failure of plasma 17-OH-corticosteroid levels to rise following the intravenous administration of corticotropin (11).

M.K. and R.L. both exhibited progressive increases in the quantity of urinary 17-ketosteroids and corticosteroids throughout the course of each pregnancy (Table 1). These observations are in agreement with those reported by Samuels *et al.* (1), Venning (2) and Knowlton *et al.* (3, 4). The level of free plasma 17-OH-corticosteroids[1] also rose during pregnancy

[1] Blood specimens were obtained eight to twelve hours after the last dose of cortisone.

(Table 1), but in contrast to normal pregnant women, neither M.K. nor R.L. showed a plasma corticosteroid response to corticotropin (ACTH) (11). During gestation their requirements for salt (5–6 Gm. per day) and for cortisone (15–25 mg. per day), were neither diminished nor increased (6).

Three groups of more specific data failed to provide evidence for the secretion of certain adrenocortical steroids by these 2 patients late in the course of pregnancy: 1) Paper chromatography was performed upon the ethylacetate extract of a pool of 80 ml. of plasma obtained from R.L. in the

TABLE 1. PLASMA AND URINARY STEROID STUDIES IN 2 WOMEN WITH ADDISON'S DISEASE DURING PREGNANCY

	I Trimester	II Trimester	III Trimester	Post partum
Patient R.L.				
Urinary 17-ketosteroids, mg./24 hrs.		6.5– 7.7	9.5–15.9	1.7
Urinary 17-OH-corticosteroids, mg./24 hrs.		6.0–10.6	9.3–19.8	3.0
Plasma 17-OH-corticosteroids, μg./100 ml.	3	14–20	8–22*	
Plasma hydrocortisone (chromatography)			0	
Urinary aldosterone, μg./24 hrs.			0.3– 0.4	
Patient M.K.				
Urinary 17-ketosteroids, mg./24 hrs.	2.5–4.8	4.9– 9.9	14.1–23.4	3.8
Urinary 17-ketosteroids (chromatography)			0	
Urinary 17-OH-corticosteroids, mg./24 hrs.	4.2–6.5	1.7–14.8	10.4–21.8	2.7
Plasma 17-OH-corticosteroids, μg./100 ml.	7–11	6–23	12–23*	22–0

* Administration of ACTH (4 hours, i.v.) elicited no response in plasma 17-OH-CS concentration. Values before and after ACTH were 20 μg. and 6 μg. per 100 ml. respectively in Patient R.L., and 23 μg. and 14 μg. respectively in Patient M.K. who also showed no fall in circulating eosinophils (40/cu. mm. before, and 102/cu. mm. after ACTH).

third trimester. Individual blood samples for the plasma pool were withdrawn twelve hours following the last dose of cortisone in order to allow adequate time for disappearance from plasma of exogenous steroid. Chromatography (chloroform-methanol-formamide (12)) failed to reveal an ultraviolet light-absorbing area in the hydrocortisone position. This technic uniformly detects such an area in similar or smaller quantities of normal plasma. The elevated plasma 17-OH-corticosteroid levels described (Table 1) would therefore seem to be related to a chromogen other than hydrocortisone. 2) Gradient elution chromatography (13) was used to fractionate 17-ketosteroids (*i.e.*, Zimmermann chromogen) in urine obtained from Patient M.K. near term. Infrared spectroscopy was carried out on the chromatographic peaks, but no identifiable 17-ketosteroid could be found. Specifically, analysis failed to show a detectable quantity of 11-oxygenated 17-ketosteroids, that is, of the characteristic 17-ketosteroids resulting from the degradation of exogenous, and presumably of endog-

* John and Mary R. Markle Scholar in Medical Science.

enous hydrocortisone. The technic is sensitive at a level as low as approximately 70 micrograms of a given 17-ketosteroid. 3) Bio-assay for salt-retaining activity was performed upon extracts of two 24-hour urine specimens obtained from Patient R.L. late in the third trimester (14). The specimens were collected during a period of hypertension and edema (resembling pre-eclampsia clinically, but not accompanied by proteinuria) which persisted until several days after delivery (15). Assay showed 0.3 and 0.4 microgram respectively of aldosterone per twenty-four hours. These values are regarded as tantamount to zero (14). It should be pointed out that the preparation of urinary extracts for bio-assay which was used at the time (1955) did not include purification of urine extracts by chromatographic means (14). The values would therefore be expected to be falsely high rather than falsely low. The failure to detect aldosterone in a pregnant Addisonian confirms the observation of Baulieu *et al.* (8) but is not in accord with the tentative identification of aldosterone in the urine of an adrenalectomized pregnant woman which has been reported by Laidlaw and co-workers (16). No explanation for the discrepancies is forthcoming at this time.

Within the limits of sensitivity of the methods used in the present study, it can be stated that no evidence for aldosterone secretion could be found in Patient R.L., and that no evidence for hydrocortisone secretion was obtained from either Patient M.K. or Patient R.L. No statement can be made concerning the presence or absence of other adrenocortical steroids not searched for, or not detectable by the technics employed (*e.g.*, corticosterone). It seems permissible to conclude that the apparent increase in urinary steroid values in both patients and the occurrence of a toxemia-like episode in one (R.L.) were probably not related to the secretion of 2 major adrenocortical hormones during pregnancy.

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UNUSUAL PLASMA 17-KETOSTEROID PATTERN IN A BOY WITH CONGENITAL ADRENAL HYPERPLASIA AND PERIODIC FEVER

TO THE EDITOR:

THE principal neutral 17-ketosteroids in the peripheral plasma of a normal human adult have been characterized as androsterone and dehydroepiandrosterone (1). Following this observation the question was raised as to why there should be so much etiocholanolone in the urine of normal adults and yet little or none in the peripheral plasma. In 1957 Kappas *et al.* (2) reported that repeatedly crystallized etiocholanolone

caused an acute pyrogenic and inflammatory reaction when injected intramuscularly in man. The manifestations following these injections were: fever to 103°F., shaking chills, weakness, muscular aches and pain, anorexia, sweating and severe headache. Of numerous other steroids tested, only pregnane-3α-ol-20-one produced such a pyrogenic reaction. Similar findings with etiocholanolone were also reported by Segaloff *et al.* (3).

In 1958 Bondy *et al.* (4) demonstrated the presence of unconjugated etiocholanolone in the plasma of 2 men with periodic fever, malaise, nausea and myalgia. In their Patient W. S. no etiocholanolone could be detected in the plasma between attacks. During attacks he excreted in the urine only etiocholanolone, whereas between attacks he excreted 17-ketosteroids in the normal pattern. Both patients showed an exacerbation of symptoms when ACTH was given. Patient W. S. was found to have an elevated urinary excretion of 17-ketosteroids but a normal concentration of plasma 17-hydroxycorticosteroids; there was a decrease in the urinary 17-ketosteroids when corticosteroid therapy was given. In this patient there had been no history of precocious sexual development, such as classically occurs in congenital adrenal hyperplasia. Bondy *et al.* suggested that the attacks of periodic fever in their 2 patients were a result of the presence of unconjugated etiocholanolone in the plasma.

The present report concerns a 13½-year-old boy with congenital adrenal hyperplasia who had had periodic fever since early life. Precocious sexual development was discovered when he entered kindergarten. Details of his earlier history were published in 1956 (5). Since infancy he had exhibited frequent episodes of fever, prostration, abdominal pain, headache, flushing and conjuctivitis. Later it was found that hypotension was a component of these paroxysmal attacks. In 1955 the episodes suddenly ceased, and there have been no recurrences since that time, although the conjunctivitis has persisted. The cessation of attacks appeared to be unrelated to corticosteroid therapy. In 1953, when the patient was still having recurrent paroxysmal attacks, plasma 17-hydroxycorticosteroid levels were found to be low (3.2 μg. per 100 ml.); therefore 17-ketosteroid determinations were made on aliquots of the same specimen of plasma by both the technique of Migeon and Plager (1) and that of Gardner (6).[1] The plasma 17-ketosteroid level was greatly elevated for a child his age (Fig. 1). The graph demonstrates an interesting discrepancy in the data obtained by the 2 techniques. The method of Migeon and Plager, which specifically measures

[1] When normal young adults were tested, a mean value of 58.5 μg. per 100 ml. of plasma for androsterone+dehydroepiandrosterone was obtained by the method of Migeon and Plager (1), and a mean value of 61.0 μg. per 100 ml. for total plasma 17-ketosteroids by the method of Gardner (6, 7).

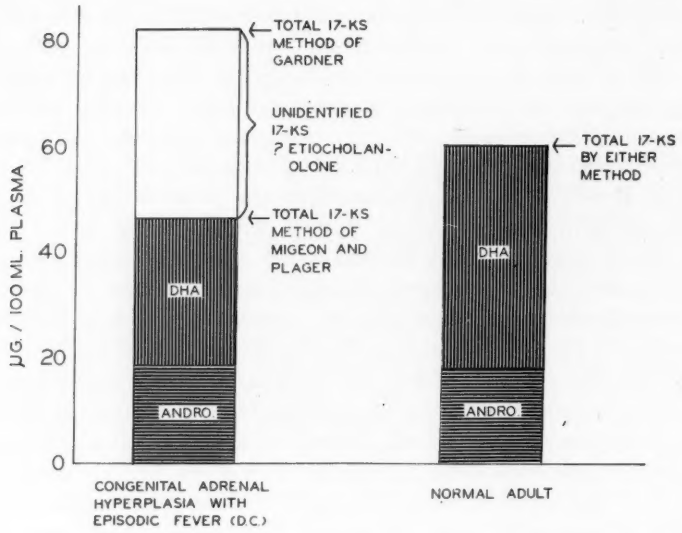

FIG. 1. Plasma neutral 17-ketosteroid pattern in a patient with congenital adrenal hyperplasia and periodic fever contrasted with the pattern found in a normal adult.

androsterone and dehydroepiandrosterone in plasma, yielded average values of 45 μg. per 100 ml. for the additive concentration of these 2 steroids. The method of Gardner, which is less specific and estimates total plasma 17-ketosteroids, yielded average values of 82 μg. per 100 ml. Although there was no ready explanation for this discrepancy when the measurements were made in 1953, the new findings of Bondy *et al.* now strongly support the possibility that the "missing" plasma 17-ketosteroids were in fact etiocholanolone. Since our patient has had no further paroxysmal episodes for several years, it is not possible to make a more direct confirmation of this hypothesis at this time.

SUMMARY

The peripheral plasma of a boy with congenital adrenal hyperplasia and episodic fever was analyzed for 17-ketosteroids by a technique specific only for dehydroepiandrosterone and androsterone and also by a technique which estimates the level of total plasma 17-ketosteroids. The results with the latter method exceeded the results with the former method by 37 μg. per 100 ml. The discrepancy between the values obtained suggests the presence of a 17-ketosteroid other than dehydroepiandrosterone and androsterone in the plasma of this patient. In the light of the recent find-

ings of Bondy *et al.* it is postulated that the "missing" plasma 17-ketosteroid in this patient may have been etiocholanolone.

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August 2, 1958.

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ADRENAL HYPERRESPONSIVENESS RESULTING FROM PROLONGED CORTICOTROPIN THERAPY*

TO THE EDITOR:

Most patients with Cushing's syndrome associated with bilateral adrenal hyperplasia respond to the administration of corticotropin (ACTH) with inordinately large increments in plasma (1) and urinary (2) levels of 17-hydrocorticosteroids (17-OHCS). Such hyperresponsiveness is inter-

* This study was supported in part by a research grant (No. RG-4998) from the National Institute of Arthritis and Metabolic Diseases, Public Health Service.

pretable equally well as indicating 1) an intrinsic adrenal lesion, or 2) chronic overstimulation of previously normal adrenal glands. If the latter interpretation were correct, patients with "iatrogenic Cushing's syndrone" resulting from long-term ACTH therapy would be expected to respond to adrenal stimulation in a manner comparable to that found in the naturally occurring disease. Although Christy *et al.* (1) expressed the view that "the concept of an intrinsic adrenal cortical lesion is perhaps inadequate to explain the pathogenesis of bilateral adrenal hyperplasia," they were unable to find instances of adrenal hyperresponsiveness in patients being treated with ACTH. However, it may be that the duration and intensity of corticotropin therapy necessary to induce adrenal hyperresponsiveness is only rarely approached clinically. One of the patients reported here was subjected to intensive long-term administration of corticotropin. His response to ACTH infusions, in contrast to that of 2 other patients not previously exposed to ACTH, suggests strongly that he exhibited corticotropin-induced adrenal hyperresponsiveness.

CASE REPORTS

Mr. A.D. On December 5, 1955, this 35-year-old business executive was admitted to the Presbyterian Hospital for treatment of optic atrophy secondary to retrobulbar neuritis. The patient first noted decreased visual acuity three and a half years previously, following a "head cold." Multiple tooth extractions, typhoid-induced hyperthermia, and salicylate, tolazoline or nicotinic-acid therapy were all tried without effect. Corticotropin therapy, as prescribed by Wolfson (3), was instituted on February 15, 1954. Initially, there was dramatic improvement in vision, but soon progressive deterioration ensued so that visual acuity became greatly reduced. Despite the lack of response, corticotropin (as ACTH-gel, intramuscularly) was continued. At the time of the admission to the hospital, the patient had been under continuous treatment for 658 days. Dosages varied from as much as 400 units daily to 60 units on alternate days; the latter was the schedule employed for one month preceding hospitalization. Supportive medication included varying dosages of desiccated thyroid, potassium chloride and calcium lactate.

On physical examination, the patient was found to be a highly intelligent, slightly obese adult male who did not appear ill. Old and new acneiform lesions were widespread over the back, shoulders and face. The typical "moon-face" was evident, and there was increased pigmentation, particularly in the exposed areas of the skin and in the periorbital regions. Vision was 15/200 in both eyes. Blood pressure was 132/92 mm. Hg. The remainder of the physical findings were unremarkable. Results of routine hematologic and urine examinations were normal. Values for blood nonprotein nitrogen, blood sugar, serum cholesterol, chloride, carbon-dioxide combining power, sodium, potassium, calcium, phosphorus, and alkaline phosphatase were all in the normal range. The basal metabolic rate was −1 per cent.

Intravenous infusions of ACTH (40 units per liter) were begun on December 6, 1955 and continued through December 14, 1955 (Fig. 1). With moderate salt restriction and potassium chloride supplement, this regimen was well tolerated. The patient's admission weight of 168 pounds remained constant, and the blood pressure fell slightly. There was no change in visual acuity. Following the patient's discharge from the hospital on De-

cember 19, 1955, he was given decreasing doses of ACTH-gel as single intramuscular injections, until January 15, 1957, when corticotropin therapy was permanently discontinued.

It is of interest to compare the urinary corticosteroid levels of Mr. A. D. (Fig. 1) with those of 2 other patients:

Mrs. E.P. was a 35-year-old married nurse who was admitted to the Presbyterian Hospital on June 26, 1958 for treatment of retrobulbar neuritis. She had been in good health until twelve days previously, when she noted impairment of vision in the left eye. There were no constitutional symptoms and the right eye remained unaffected throughout her course. One week prior to admission her physician prescribed prednisone, 5 mg. per day. Vision in the left eye continued to deteriorate, so that three days before admission the dosage of prednisone was increased to 20 mg. per day. Physical findings were unremarkable, except that vision on the left was reduced to simple light perception. ACTH was administered intravenously as indicated in Figure 2. On the third day of therapy vision began to improve and at the time of discharge on July 11, 1958, visual acuity had returned to normal.

Mrs. L. K., a 33-year-old housewife, had suffered from severe asthma for many years. For two years prior to admission to the Presbyterian Hospital on November 27, 1955, she had been treated with large doses of a variety of adrenal corticoids. The schedule of intravenous corticotropin therapy in the hospital is charted in Figure 3. Urine samples were collected sporadically, and for this reason the excretion of 17-OHCS is plotted as

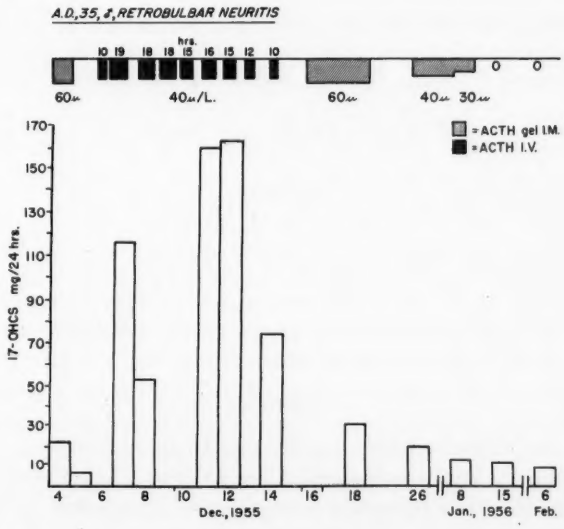

FIG. 1. Pattern of 17-OHCS excretion in a patient previously treated for two years with ACTH. The 24-hour urine collections represented in this and the following figures were started at 8 A.M. The black rectangles are plotted as an indication of the time of day the infusion was administered; the duration of each infusion in hours in noted above each rectangle. The numbers on the abscissa denote the day of the month.

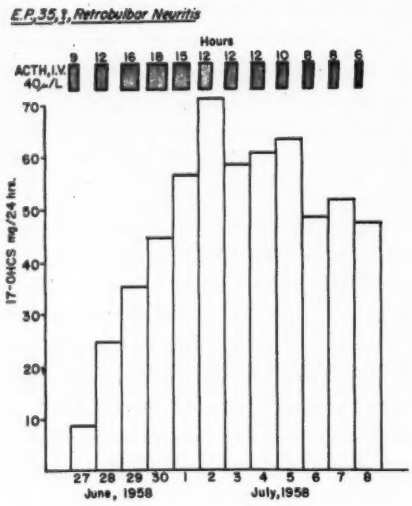

FIG. 2. Pattern of 17-OHCS excretion in a patient previously given only a brief course of prednisone.

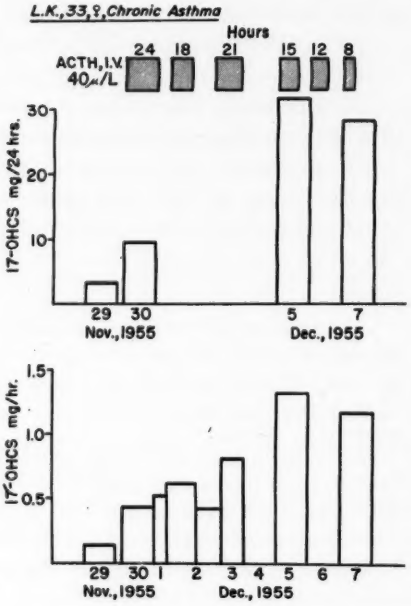

FIG. 3. Pattern of 17-OHCS excretion in a patient previously given a long-term course of corticosteroids in large doses.

milligrams per hour as well as milligrams per day. The patient improved considerably during hospitalization.

METHODS

The urinary excretion of 17-OHCS in all 3 patients was determined by the method described by Silber and Porter (4), following incubation of the urine samples with β-glucuronidase. Urinary 17-ketosteroids were determined by the method of Drekter *et al.* (5). The duration of the ACTH infusions is indicated in the figures. Infusions were given at the slowest rate that would maintain patency of the intravenous needle. Not more than 1500 ml. was given in any single period.

COMMENT

Although no formally timed studies of adrenocortical responsiveness (1, 2) or capacity (6) were performed, it is evident that Patient A.D., previously subjected to inordinately intensive and protracted ACTH therapy, responded to intravenous administration of corticotropin with dramatic increases in 17-OHCS excretion. These 17-OHCS values were well within the range of what would be expected in a patient with naturally occurring Cushing's disease. Furthermore, the 24-hour excretion of 17-ketosteroids increased from 9.4 mg. on the day prior to the beginning of intravenous

therapy to 65.9 mg. on the day when 17-OHCS excretion had risen to 159.5 mg. per twenty-four hours. Following discharge from the hospital, the patient was given decreasing doses of ACTH-gel. When corticotropin therapy was finally discontinued, 17-OHCS excretion had fallen to normal levels.

No systematic studies of the response to intensive administration of ACTH in normal subjects are available, but the striking findings in Mr. A. D. may be contrasted with those in the other 2 patients. Mrs. E. P. had a similar illness but had not been treated with corticotropin prior to admission. The unimpressive response of 17-OHCS excretion on the first day of ACTH therapy may be attributed to the prior short course of treatment with prednisone. Urinary 17-OHCS levels rose progressively, reaching a maximum of 71.1 mg. per twenty-four hours on the sixth day of therapy. Similarly 17-ketosteroid excretion rose from an initial value of 12.2 mg. per twenty-four hours to a maximum of 78.0 mg. on the ninth day.

The findings in Mrs. L. K. are representative of the other end of the spcetrum of adrenal responsiveness. She responded to intravenous ACTH with a steady increase in the excretion of corticosteroids, but the magnitude of the response was greatly diminished. The 24-hour excretion of 17-OHCS rose from 3.5 mg. to a maximum of 31.8 mg. on the sixth day of treatment.

The intensity of corticotropin therapy can be gauged roughly as the mean duration of ACTH infusion from the onset of therapy to the day of maximal response. These values, calculated as hours of infusion (exposure to ACTH) per day, were 15.8, 13.7 and 13.0 for Patients A. D., E. P. and L. K., respectively. Thus, Patient A. D. was treated somewhat more intensively than Patients E. P. or L. K., but it seems unlikely that these differences could account for the observed differences in the magnitude of 17-OHCS excretion.

A comment on the relative merits of plasma and urine determinations in assessing adrenocortical function seems pertinent. The Salt Lake City group (6) has reported that the increase in plasma 17-OHCS concentration which occurs during a six-hour infusion of 25 units of ACTH is a reflection of adrenocortical capacity. However, these workers have also carefully focussed attention on extra-adrenal factors (7) which may strongly influence *plasma levels*, and hence the determination of adrenocortical capacity as defined here. On the other hand, urinary corticosteroid excretion (in subjects with normal kidneys) is in large part a function of the *total* adrenocortical output, and is therefore less susceptible to misinterpretation. Since there was no evidence of abnormal renal function in the patients reported here, it is unlikely that extra-adrenal factors would distort the values for urinary 17-OHCS excretion to any great extent.

One further point deserves mention. Eik-Nes *et al.* (6) noted that meas-

urements of adrenocortical capacity are valid only when adrenal activity is in a steady state. It could be argued that in the patients reported here, the repeated daily infusions of corticotropin provoked changes in adrenocortical capacity. This may well be true. If so, it still is of interest that Patient A. D. had the "capacity" to increase his adrenocortical capacity to a much greater degree than the other 2 patients.

It seems reasonable to conclude that the increased magnitude of adrenocortical response in Patient A. D. was the result of augmented adrenocortical capacity produced by chronic exogenous stimulation of the adrenals. Similarly the hyperresponsiveness of patients with Cushing's syndrome associated with bilateral adrenal hyperplasia could be simply a reflection of chronic endogenous stimulation of the adrenal glands.

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September 11, 1958

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The Endocrine Society

THE 1959 ANNUAL MEETING

The Forty-First Annual Meeting of The Endocrine Society will be held in the Haddon Hall Hotel, Atlantic City, New Jersey, Thursday, Friday, and Saturday, *June 4, 5 and 6, 1959.*

The Chairman of Local Arrangements is Dr. Matthew Molitch.

Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily, and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 5, at 7:30 P.M. preceded by cocktails at 6:30 P.M.

All members are urged to make their hotel reservations immediately. The Chalfonte-Haddon Hall Hotel will hold 300 bedrooms for members until May 1, 1959, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations now directly with the hotel, advising them of time and date of arrival and departure. Avoid disappointment.

Final program, membership card and advance registration forms will be sent on May 1, 1959 to members whose current dues have been paid.

Those wishing to present papers, which will be strictly limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Frank Engel, Duke Hospital, Durham, North Carolina, not later than *February 1, 1959. It is imperative that the abstracts be informative and complete with results and conclusions*—not a statement that those will be presented at the meeting—in order that they may be of reference value and suitable for printing in the program. The reading and processing of approximately two hundred abstracts submitted each year and the compiling of the program from these involves a tremendous amount of time and effort on the part of the Program Committee. The Council requests that the authors adhere strictly to the following rules when submitting abstracts, otherwise they cannot be considered:

1. *It is assumed that abstracts submitted for this program have not and will not be submitted elsewhere.*
2. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the

text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.

3. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of the nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be written after his name.
   Line 3. Institution of origin and city in which institution is located.
4. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
5. Abstracts should be letter perfect, since there will be no opportunity for proof reading by the authors.

THE 1959 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

FRED CONRAD KOCH AWARD

During the past year a substantial legacy has been bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is to be the highest honor of The Endocrine Society and is to be represented by a medal that is to be known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award will replace the Medal of The Endocrine Society which was established in 1954 and presented to Dr. Carl R. Moore in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957 and Dr. I. L. Chaikoff in 1958. The Medal of the Endocrine Society replaced the E. R. Squibb Award which was formerly the highest honor bestowed by the Society.

Past recipients of the Squibb Award were Dr. George W. Corner in 1940, Dr. Philip E. Smith in 1941, Dr. Fred C. Koch in 1942, (no award in 1943), Dr. E. A. Doisy in 1944, Dr. E. C. Kendall in 1945, Dr. Carl G. Hartman in 1946, Drs. Carl F. and Gerty T. Cori in 1947, Dr. Fuller Albright in 1948, Dr. Herbert M. Evans in 1949, Dr. C. N. H. Long in 1950, Dr. J B. Collip in 1951, Dr. James H. Means in 1952 and Dr. David Marine in 1953.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr. Monte A. Greer. Prior to 1952 the Award was $1,200. It has now been increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

ELEVENTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE UNIVERSITY OF CALIFORNIA SCHOOL OF MEDICINE AND DEPARTMENT OF CONTINUING MEDICAL EDUCATION

SAN FRANCISCO, CALIFORNIA

November 9 through 13, 1959

For information concerning the program and registration, write to *Seymour M. Farber, M.D.*, Assistant Dean in charge of Continuing Medical Education, University of California Medical Center, San Francisco 22, California.

The registration fee is $100. Residents and Fellows will be admitted for a reduced fee of $30. Those wishing to reserve rooms should communicate directly with the Hotel Sir Francis Drake, San Francisco.

The American Goiter Association

THE 1959 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the Drake Hotel, Chicago, Illinois, on *April 30, May 1 and 2, 1959.*

THE AMERICAN SOCIETY FOR THE STUDY OF STERILITY

The Fifteenth Annual Meeting of The American Society for the Study of Sterility will be held at the *Shelburne Hotel, Atlantic City, N. J., April 3–5, 1959.* Persons interested in attending this meeting should apply for advance registration and a complete program to the Secretary, *Dr. Herbert H. Thomas, 920 South 19th Street, Birmingham 5, Alabama.* The President of The Society for 1959 is Dr. Sheldon Payne, Los Angeles, California; Chairman of the Program Committee, Dr. Edward C. Reifenstein, Jr., New Brunswick, N. J.; and Chairman of the Local Arrangements Committee, Dr. Luigi Mastroianni, Jr., New Haven, Conn. Immediately following this meeting, the Annual Clinical Meeting of The American College of Obstetricians and Gynecologists will also be held (April 6–8) in Atlantic City at the Municipal Auditorium.

The program will cover such subjects as the genetic hazards of medical radiation, infertility in animals, the use of estrogens and other steroids to induce ovulation, endocrine control of passage of sperm and ova through the female genital tract, the metabolism of progesterone and related compounds, maintenance of pregnancy with progestogens, associated genital anomalies, the use of androgens and other steroids in pregnancy and in treatment of sterility, treatment of endometriosis, study of cervical mucus, spermigration and uterine contraction patterns, damage to seminiferous epithelium by heat, hypnosis in the treatment of infertility, thyroid function, pathophysiology of the pubococcygeus muscle, hydrotubation, and culdoscopic technic.

BOOK REGISTER

Die medikamentöse Behandlung der Schilddrüsenerkrankungen. By PROF. DR. W. GRAB, Director of the Pharmacological Institute of Justus-Liebig University, Giessen, and PROF. DR. K. OBERDISSE, Director of the Second Medical Clinic and Polyclinic of the Academy of Medicine, Düsseldorf, Germany. This book deals with various newer aspects of the medical management of thyroid diseases. Part I covers the pharmacology. The subjects include the functional relationships of the thyroid, the physiology of the healthy gland, the pathophysiology of thyroid disorders, the morphology of the thyroid, the disposition of iodine in the body, the fundamental action of the thyroid hormones, the effect of a healthy *vs.* a diseased thyroid gland on the organism, therapy with thyroid hormones, iodine, antithyroid substances, and other agents, and a pharmacological and chemical appraisal of methods used for determination of various thyroid hormones and related compounds. Part II covers the clinical aspects, including discussions of various diagnostic laboratory methods, the management of hyperthyroidism, malignant exophthalmos, thyroiditis, goiter and hypothyroidism; there is a special section on hypermetabolism and hypometabolism in old people; 285 pages; 55 figures; 14 tables; 1959. Georg Thieme Verlag, Stuttgart. In the U.S.A. and Canada, Intercontinental Medical Book Corporation, New York 16, N.Y. Price DM 39.60. $9.40.

Lipidoses, Diseases of the Intracellular Lipid Metabolism. Edition 3, revised and enlarged. By SIEGFRIED J. THANNHAUSER, M.D., Ph.D., Clinical Professor of Medicine Emeritus, Tufts College Medical School, Boston; Consulting Physician, Pratt Diagnostic Hospital, New England Medical Center, Boston. There are chapters on the physiology and chemistry of lipid metabolism, hyperlipemia, xanthomatoses, Gaucher's disease (reticular and histiocytic cerebrosidosis), Niemann-Pick's disease (reticular and histiocytic sphingomyelinosis) and a supplement on infantile amaurotic idiocy and gargoylism. The section on xanthomatoses is by far the largest and contains subsections on hypercholesteremic xanthomatoses per se and secondary to liver disease, hyperlipemia with secondary eruptive xanthoma, and normocholesteremic xanthomatoses; 600 pages; 126 figures; 50 tables; 1958. Grune & Stratton, Inc., 381 Fourth Avenue, New York 16, N.Y. Price $19.75.

Water and Electrolyte Metabolism in Relation to Age and Sex. Vol. IV of Ciba Foundation Colloquia on Ageing, edited by G. E. W. WOLSTENHOLME, M.A., B.Ch. and CECILIA M. O'CONNOR, B.Sc. Twenty-seven authorities participated in this colloquium. The material covers the entire ageing process from birth onward, and reveals some interesting variations in water and electrolyte composition at different ages. A partial table of contents is as follows: Development of physiological regulation of water content; cellular aspects of the electrolytes and water in body fluids; glandular secretion of electrolytes; hormonal aspects of water and electrolyte metabolism in relation to sex and age; body water compartments throughout the life span; clinical consequences of the water and electrolyte metabolism peculiar to infancy; the effect of age on the electrolytes in the red blood cells of different species; the development of acid-base control; the role of the kidney in electrolyte and water regulation in the aged; age and renal disease; and water and electrolyte metabolism in congestive failure; 327 pages; 85 illustrations; 1958. Little, Brown and Company, Boston, Massachusetts. Price, $8.50.